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DNA IDENTIFICATION

HEARING

BEFORE THE

SUBCOMMITTEE ON THE CONSTITUTION

OF THE

COMMITTEE ON THE JUDICIARY
UNITED STATES SENATE

ONE HUNDRED FIRST CONGRESS

FIRST SESSION

ON

GENETIC TESTING AS A MEANS OF
CRIMINAL INVESTIGATION

MARCH 15, 1989

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DNA IDENTIFICATION

WEDNESDAY, MARCH 15, 1989

U.S. SENATE,
SUBCOMMITTEE ON THE CONSTITUTION,
COMMITTEE ON THE JUDICIARY,
Washington, DC.

The subcommittee met, pursuant to notice, at 10:08 a.m., in room SD-226, Dirksen Senate Office Building, Hon. Paul Simon (chairman of the subcommittee) presiding.

Also present: Senator Specter.

Senator SIMON. The subcommittee hearing will come to order.

First, I am going to put a statement into the hearing record by Senator Orrin Hatch, my colleague on the subcommittee here.

[The statement of Senator Hatch follows:]

Statement of Senator Orrin G. Hatch
Hearing on DNA Identification
Subcommittee on the Constitution
March 15, 1989

Thank you, Mr. Chairman. I commend you for holding this hearing today. Given the tremendous potential of genetic testing as a means of criminal identification, I believe that we should very carefully examine the issues surrounding its use.

I am informed that molecular biologists have developed various tests in which DNA is examined and mapped to determine the sequencing of nucleotides. Since identical DNA patterns are shared only by identical twins, DNA typing has great potential for specific identification of individuals. This offers great promise in civil matters such as determination of paternity in child support cases. It offers even greater promise in criminal matters, where it has the potential to improve substantially the capabilities of forensic investigations.

This is particularly so for the most tragic crimes. Rapists and murderers may not leave recoverable fingerprints. They do, however, often leave evidence such as semen, blood, or hair, which may provide the basis for identification through DNA typing. DNA thus offers law enforcement officials the possibility of going much further toward criminal identification, with much more accuracy, than conventional methods allow.

Just as important as DNA's potential for <u>in</u>culpation, however, is its potential for <u>ex</u>culpation. DNA typing can help convict the guilty, but it can also protect the innocent. For example, in one of the earliest cases where DNA typing was used, a multiple rape-murder in England, a suspect was cleared when DNA typing of his blood and of semen taken from the victims' bodies showed two distinct patterns.

My home state of Utah has hosted some pioneering research and development of DNA typing techniques. Dr. Ray White, of the Howard Hughes Medical Institute research laboratory at the University of Utah in Salt Lake City, is well-known in the forensic science community as the developer of the probes used in the most common DNA typing test.

Mr. Chairman, before concluding, I wish to mention my particular interest in the constitutional issues surrounding DNA testing. As one who believes that the individual needs protection from the government as much as he or she needs protection by the government, I wish to ensure that DNA testing, as with other elements of criminal investigations, is conducted in a constitutionally sound manner. The Fifth Amendment to our Constitution states that "No person . . . shall be compelled in any criminal case to be a witness against himself." Although the Supreme Court has not yet considered a case involving DNA evidence, its decision in Schmerber v. California, 384 U.S. 757 (1966), regarding a blood test, seems to indicate that DNA typing would be considered consistent with this highly important protection against self-incrimination. In Schmerber, the Court ruled that taking a blood sample from a suspect does not compel him to testify against himself or otherwise provide testimonial or communicative evidence.

The Fourth Amendment states, "The right of the people to be secure in their persons, houses, papers, and effects, against unreasonable searches and seizures, shall not be violated, and no Warrants shall issue, but upon probable cause . . ." Under Schmerber, so long as probable cause exists to take a sample, DNA typing appears consistent with the requirements of the Fourth Amendment as well.

Mr. Chairman, I look forward to a thorough review of the testimony presented today.

OPENING STATEMENT OF HON. PAUL SIMON, A U.S. SENATOR FROM THE STATE OF ILLINOIS

Senator SIMON. I apologize in advance to the witnesses. We are marking up a bill in another committee on which I serve. We may have to from time to time take temporary recesses for me to go to that other committee hearing. But it is just down the hallway, so they should be very brief recesses, if we have to take them.

We are holding a hearing on something that has the potential for being a major breakthrough in crime identification and in work in solving crimes, and also proving the innocence of people. It could be a major new weapon in the fight against crime; and we are talking about the use of DNA identification.

I am a layman, so you are going to have to explain all this in very simple terms. But as I read about what is happening here, it is a form of identification that is really remarkable where the odds of making a mistake are 1 in 3 billion. If the information I have is correct, it can become a remarkable breakthrough.

We face practical problems of implementation. We face practical problems of cost, and we are here to listen to what the impact of all of this may be and how we move ahead. It is an area where I think we can see some fairly dramatic changes in law enforcement in this country. Obviously, the uses of DNA go far beyond law enforcement, but we are focused only on this one aspect of it here in this committee.

I am very pleased to have as the first panel of witnesses Prof. James Starrs, of the National Law Center of George Washington University, and Dr. Joseph Peterson, the head of the Department of Criminal Justice at the University of Illinois at Chicago.

Professor Starrs, we will hear you first.

PANEL CONSISTING OF JAMES E. STARRS, PROFESSOR OF LAW AND FORENSIC SCIENCES, GEORGE WASHINGTON UNIVERSITY, WASHINGTON, DC; AND JOSEPH L. PETERSON, ASSOCIATE PROFESSOR AND DEPARTMENT HEAD, DEPARTMENT OF CRIMINAL JUSTICE, UNIVERSITY OF ILLINOIS AT CHICAGO, CHICAGO, IL

STATEMENT OF JAMES E. STARRS

Professor STARRS. Thank you, Senator. I bid the Senator a very unfateful, but good, Ides of March.

My name is James E. Starrs. I hold a joint professorial appointment at the George Washington University. I am a professor of law and a professor of forensic sciences. I am also, it is proper to add, a consultant to Cellmark Diagnostics, a commercial leader in the forensic uses of DNA profiling, called by them DNA fingerprinting.

Although I am, by profession, more a lawyer than a scientist, and although I am concededly not a molecular biologist, I understand, as an informed nongeneticist, it is my charge today to give you a hop, skip, and a 5-minute jump through DNA profiling for forensic purposes.

The subject that I address on this occasion is from the perspective of the lawyer or other nonscientist and not from the scientist. The topic is one of much complexity and, indeed, as this slide indi-

cates, of much bewilderment, so much so that press reports on DNA's legal uses have been replete with myths and fictions.

All of the DNA in each person is not different from all of the DNA of every other person. Only a small fragment of each person's DNA is individual to that person and none other. Our common humanity would seem to make that point self-evident.

Although DNA profiling uses more biological samples than does traditional blood typing for genetic markers, still DNA profiling does have its limits. It requires nucleated cells, and crime scene biological evidence, like hair, for example, is not always of that variety.

The DNA molecule, called a macromolecule because when stretched out it extends up to a meter-and-a-half in length comprising some 3 to 5 billion base pairs, is composed of two helical, spiral, complementary strands. It is this complementary nature of these strands that gives DNA its potential for clinical and forensic applications.

The DNA molecule has four interlocking chemical components. You see them here. I address them as A, T, G, and C. We know that A always links with T, and that G necessarily pairs with C.

Consequently, if we can synthesize a known single-stranded fragment of DNA, that fragment can then be used as the template, as a probe, to locate its complementary sequence in a paternity or crime scene situation. This complementary pairing reflected in this slide is the warp and woof of DNA profiling.

The DNA molecule, based on our present and ever-expanding knowledge of it, can be further evaluated according to its coded and noncoded regions. The coded regions, called exons, are instrumental in elemental life-giving processes. The noncoded regions, sometimes called introns, perform functions that are only vaguely, if at all, comprehended at this time. It is the noncoded regions—I emphasize, the noncoded regions—that are the focus for individualization by DNA profiling.

The process of DNA profiling, complex as it is, involves a number of individual steps that have become quite routine in the clinical nonforensic setting. The forensic uses of these techniques are therefore neither new nor unsubstantiated by scientific testing.

The first step is the extraction process, both to obtain a biological sample such as semen and to isolate the DNA from it. The next step involves the cutting of the DNA at known sites in the molecule, employing the snipping powers like a scissors of restriction enzymes.

The cut-up DNA is then spotted in an agarose gel something of the consistency of jello, which is then charged with electricity, causing the migration of the restriction fragments that have been cut up, the smaller moving further and faster, the larger being more cumbrous, less so. This migration causes the fragments to separate according to their size.

The remainder of the technology is aimed at simply visualizing the migration so that it can be read and interpreted. The migrated fragments are first transferred to a more permanent and usable medium, a nylon membrane, by a process called Southern blotting. The fragments are denatured, so that what was once a double helix is now single-stranded and ready to be paired off.

Cloned or chemically synthesized probes of known sequences, which probes are labeled with radioactive isotopes, are then added to the nylon membrane to seek out and to pair up with their complementary bases.

The nylon membrane is then brought into close proximity to an x-ray film which, when developed, reveals the migrated restriction fragments in bands such as you see on the right, not unlike the coding of prices on items sold in today's supermarkets.

This autorad, as it is called, or developed x-ray film, is then read manually, generally, and, in the instance of a criminal case such as this, enables a suspect to be either identified with the highest degree of certainty or to be excluded with equal assurance.

This autorad enabled Cellmark to identify a suspect in a Florida rape murder case, with the outcome in part reliant upon this autorad and Cellmark's testimony, of a finding of guilty.

I thank you for the opportunity to make this presentation which should be interpreted as in the strongest conceivable support of the forensic uses of DNA profiling.

[Information of Professor Starrs follows:]

MATERIALS ON THE LEGAL USES

of

DNA PROFILING

by

James E. Starrs,
Professor of Law & Forensic Sciences,
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Washington, D.C. 20052

Prepared for the United States Senate, Committee on the Judiciary, Subcommittee on the Constitution, March 15, 1989.

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The Dawning of DNA in the Legal World:

A Red Sky in the Morning?

Testimony

Of

Professor James E. Starrs

Remarks on DNA prepared by James E. Starrs, Professor of Law and Forensic Sciences, The George Washington University, Washington, DC 20052, a consultant in forensic sciences as well as a consultant to Cellmark Diagnostics on the occasion of appearing before the United States Senate's Subcommittee on the Constitution of the Committee of the Judiciary.

In truth, it is a second coming*. At least in "forensics," that is. First there was fingerprinting to identify a suspect, without a shadow of a doubt, as having been present at a crime scene. And now the day of DNA (deoxyribonucleic acid) matching as an aid to law enforcement has dawned -- in a heady and convincing fashion.

Crime scenes are being scoured for biological samples like hair, blood and semen on the fervent anticipation that the genetic building block (DNA) within them will provide a direct and conclusive genetic link to a particular suspect and to none other. Burglars, rapists and murderers are being caught and convicted in ever-increasing numbers in the all-enveloping gyre of DNA matching. Nationwide the public's pulse has been quickened by media-stimulated visions of the wondrous deeds that DNA matching has and will achieve for criminal justice. Lawyers and judges, emulating a mesmerized public, are paying hurried homage at the throne of this new prince in the realm of scientific technology. By all reports some twenty or so criminal cases have been concluded at the trial level in some seven states where DNA matching has been welcomed, with nary a word of judicial dissent anywhere.

**with regrets to William Butler Yeats*

Defense lawyers are willing to concede the legal admissibility of a DNA match that all but irrefutably fingers their clients as guilty, even of a hanging offense. Witness the defense tactics in the recently concluded capital murder trial of Timothy Spencer in Arlington, Virginia where, at a pre-trial hearing, the defense seemed to be off molly-gawking somewhere when the validity of DNA matching was under scrutiny.

Other defense attorneys, unable to ferret out an expert to oppose DNA matching, have perfunctorily toyed on cross examination with the prosecution's DNA experts in a feeble effort to demonstrate the flaws of DNA matching. The rape trial of Tommie Lee Andrews in Orlando, Florida was of that dispiriting order.

Still other defense attorneys have, Henny-Penny-like, viewed DNA matching as bringing the sky to falling. In Las Vegas, it is reported that a suspect pleaded guilty to the rape of a policeman's mother after consultation with his attorney. The client was apparently goaded to throw in the adversarial towel upon learning that a court order would be sought to obtain a sample of his blood for DNA matching. No test had yet been performed; no DNA identification had yet been made and no blood had even been drawn but the accused, in terror of the dread portent of a DNA match, capitulated.

Whereas suspects and their attorneys have lacked conviction in the face of DNA matching, prosecutors have embraced it with "passionate intensity." In the capital murder trial of Timothy Spencer, the prosecution had the laboratory evidence of bodily secretions putting the accused within a population of only 13% of Virginians who could have been the contributor of the crime scene evidence. The state also had hair from the crime scene that matched that of Spencer in all microscopic characteristics. More, glass in a jacket, admittedly belonging to Spencer, was connected through its refractive index to the broken glass at the point of entry to the murder victim's home.

Add to this telling circumstantial evidence the incriminating statements Spencer made, without being coerced or cogged by the police, and his three prior burglary convictions, to which he himself testified, and the jury's decision to convict was well-nigh preordained. In light of this compelling evidence, the DNA matching that further established Spencer's presence at the murder scene was almost pleonastic, just a soupcon to fix

with certainty the jury's decision to convict.

While DNA matching has provided the impetus for prosecutorial overkill, it has left trial judges overawed by the magnitude of its scientific underpinnings. Judge Joseph Harris, presiding at the Albany, New York rape trials of George Wesley and Cameron Bailey, received a short course in human genetics and molecular biology at the foot of prosecution expert, Dr. Michael Baird.

In this back-to-school educational effort, Judge Harris revealed he was out of his depth even on the meaning of genes and chromosomes, not to mention the chemical building blocks of DNA known as base pairs (also known as nucleotides). At one point the judge's ignorance stood out in bold relief. He asked for an explanation of the "Hardy Weinberg equilibria" in such a way as to signal his misapprehension of the fact that Hardy and Weinberg were two persons, not one, whose names had been given jointly to the same principles of inheritance independently determined.

Closer to home, in the trial of Timothy Spencer, Circuit Court Judge Benjamin Kendrick listened attentively, as attentively as any intelligent layman could, to the scrupulously precise and detailed testimony of prosecution expert Dr. Michael Baird. After Dr. Baird had concluded spelling out the intricacies of each of the laboratory steps in DNA matching, Judge Kendrick, in some obvious puzzlement, asked for clarification on one point. The judge had counted only seven steps, but the expert said there were eight in all. Was not the digestion of DNA by a restriction enzyme identical to the process of separation of fragments by gel electrophoresis, the judge inquired in so many words.

A muzzled gasp was almost audible in the courtroom from the observers schooled in the rudiments of DNA matching or even in just plain instrumental analysis. But Dr. Baird kept his composure and replied with unreproving aplomb. His patience was that of the very best educator in the presence of a totally befuddled student. His honor was gently informed that the processes were distinctly different, as distinctly different one might add, as bicycling is from swimming even though in a triathalon one follows the other.

It is one thing for a trial judge to be becalmed by the scientific vastness of DNA matching. It is another for a Supreme Court justice to jump the scientific gun on a subject of such scientific mundanity as firearms identification. Justice, now Chief Justice, Rehnquist in his 1974 opinion in *Donnelly v. DeChristoforo* referred to a weapon, unearthed in a rusted condition, as having been "recently fired." In view of the settled scientific fact that there is no known scientific technique to determine that a gun has been recently fired and that nothing in any of the records in *Donnelly* disclosed the origin of this assertion, I wrote Justice Rehnquist for an explanation of the basis for his statement. His uniquely phrased and uninformative reply was that he had no "independent recollection" of the factual foundation for that statement.

Judges are at best ill-equipped to pierce the veil of scientific principles. Routinely, they muddle along, nodding in polite unawareness of the nature or demands of the science under investigation. It is only when judges are given a life or death decision-making function on the rectitude of scientific applications that trouble comes to a boil. The sequela to Judge Kendrick's scientific gaff was his admitting DNA matching as scientifically well-ensconced, according to his informed judicial lights after less than one day of scientific testimony and all of that from persons on the prosecution team.

DNA matching has not been borne into the legal world on an indifferent, slouching wind. On the contrary, its reception has been heralded, programmed and even pre-determined in the best corporate tradition. While the F.B.I. Laboratory at Quantico, Virginia has committed much of its massive resources to a crash research effort to assay the values of DNA matching for forensic purposes, three corporations have been quietly but effectively bridging the gap between the laboratory and the courtroom.

These three corporate entities, Lifecodes of Valhalla, New York, Cellmark of Germantown, Maryland and Cetus of San Francisco, all bear names redolent of an introductory course in biology, giving them a recognizable market presence. Each performs somewhat different functions and each is to a certain extent in competition with the other, particularly in the case of Lifecodes and Cellmark.

Cellmark, an offshoot of the British corporate giant, ICI Industries, lays claim to the multi-locus probe

originated by Dr. Alec Jeffreys of the University of Leicester, England. Through the use of this probe, it is possible, according to the published reports of its inventor, to discern up to fifteen bands or individual markers in the DNA of a particular person or in that from a crime scene sample. These bands then can be compared to determine whether the crime scene sample originated from the same person from whom the known sample was obtained. The more the number of matching bands, the greater the statistical likelihood that the two DNA samples under study came from the same source.

It is this ability to pinpoint, with near perfect mathematical certainty, a particular sample to a particular person that led Dr. Jeffreys to affix the label DNA "fingerprint" to his multi-locus probe. This same certainty of individualization is what has caused defense attorneys to run scared when confronted with DNA evidence as well as that which has given prosecutors just cause to gloat with uninhibited glee at the distress of the defense.

Lifecodes, which prefers to be known as situated in Valhalla rather than Westchester County, New York, employs a single-locus probe in its DNA methodology. This probe sets its sights on a particular chromosome and a very specific site or locus on that chromosome. The band that is revealed when the probe binds to the assigned locus in a biological sample whose source is known can be compared to a crime scene sample whose source is unknown. A match between them puts the two samples within a pre-determined percentage of the population who have the identical banding pattern. All of this is familiar turf to biologists since the same Mendelian principles and the same establishment of population frequencies occurs in the every day genetic markers known as ABO blood grouping.

To narrow the percentages to a precious few so as to better fix a suspect either as guilty or not, it is imperative that the Lifecodes testing not cease with one probe. The more probes, the more discriminating the result and the more the probability that the right person is in custody for the crime. Heavily freighting the Lifecodes approach is a reliance on population frequencies, independently inherited genetic markers and the laws of Mendelian inheritance. If any one of these factors is misaligned or out of sync, then the accuracy of premising guilt or innocence on the single locus matching technique is sorely out of whack.

To an extent, Cetus complements the DNA work in legal matters performed by Lifecodes and Cellmark. In a manner of speaking, it picks up where they leave off. The aftermath of the rape trial and acquittal of Marine Corporal Lindsey Scott in Quantico, Virginia illustrates what Cetus has to offer the criminal justice system through its acting as a developmental hub for applications in other spoke-type laboratories.

Corporal Scott had put forward an alibi in support of his not guilty plea. The victim had contradicted Scott's assertion of absence from the crime scene. The DNA from the biological specimens from the victim and the crime scene could, in theory at least, tell which was tale and which was truth. But the comparison of the DNA from Scott's blood was thwarted by the inadequacy of the crime scene samples to meet the needs of sample size and lack of degradation required to conduct either a single or multi-locus probing of it at Cellmark.

Thus the bell sounded for the Cetus technology to perform its scientific wizardry through the good offices of Forensic Science Associates in Emeryville, California. The DNA fragments needed to be amplified (duplicated) by a process spearheaded by Cetus known as polymerase chain reaction (PCR). Single or even multi-locus probing could then follow on the heels of this amplification. Ruefully, the reverberations from California were a knell tolling the inability of PCR to produce anything more than the same inconclusive results as Cellmark had previously reported, according to a Washington Post news story.

While the scientific testing in the Scott case was demonstrably unrewarding for legal purposes, still the situation teaches a lesson well learned. DNA matching is no carminative preparation that will enable the criminal justice system in all cases to isolate the guilty from the innocent based upon biological samples obtained from a crime scene. It is rank hyperbole to intimate or state otherwise. So much depends upon the samples retrieved for testing and the effect of the ambient circumstances upon them.

Physical evidence from a crime scene, certainly of a biological nature, lacks the pristine qualities of controlled laboratory specimens. Suffice it to say, this is an inherent flaw, at present, in DNA matching for law enforcement purposes. Moreover, a DNA technology that is founded on the antiseptic testing of controlled laboratory samples cannot validly assert its utility in the market place of crime scene contaminated samples.

The reverse side of this coin gives promise of a more well-charted future for DNA matching in the legal world. The use of DNA matching in paternity contests should be pre-eminently reliable and assuredly dispositive, certainly if the Cellmark multi-locus probes are the method of choice. But, unfortunately, such an exhilarating conclusion depends on whether both parents are available for testing and whether there are any extraneous interferences.

Observe, in this regard, the happenings in a recent paternity suit from Easton, Maryland in which I acted as consultant to the attorney for the child's mother. It was enough of a snare that the claim of paternity was disputed. It was a snorter (in Flann O'Brien's quaint phrasing of an inscrutable conundrum) of a much more perplexing order that the putative father was deceased and had literally been dead and buried for some time. A judicial order to exhume the body of the alleged father was sought but the surviving relatives protested.

In support of the exhumation order the claimant's attorneys' sought the services of Cellmark for DNA matching. Cellmark, however, flatly and unequivocally refused to conduct the requisite tests, even supposing the body were to be exhumed. The Cellmark stance was a prudent one at the time and otherwise had sound scientific backing. The literature on the effects of embalming fluids, such as formaldehyde, on the structure of DNA is notable for its scarcity. Cellmark had not at that juncture validated its probes for this usage and putrefactive and pathogenic changes in body had to be espied, all without the guidance of scientific facts or findings.

The attorneys, however, were not daunted by this rejection and they went shopping. An appeal went out to Lifecodes for a second opinion. And Lifecodes entered the lists where Cellmark feared to tread.

That one DNA laboratory will entertain an offer to test a biological sample another DNA laboratory has rejected out of hand should put a little backbone in attorneys in criminal cases who are genuinely seeking to keep the adversarial system alive in the presence of the juggernaut of DNA matching.

Women especially have just cause to acclaim the advent of DNA matching. For too many years the life of the lore in rape complaints has been that such grievances are easy to make and difficult to refute. Women who charge rape are thus put on the legal rack that at every turning accuses them of fabrication. Rape shield laws and changes in evidentiary rules requiring corroboration have acted as an effective anodyne for the discriminatory impact of such hide-bound, gratuitous legal traditions. DNA matching can do even more to guarantee that the truth will be told in rape prosecutions. But, it is to be remembered, DNA matching in its most accredited form is non-partisan, letting the conclusions fall where they impartially may. It is, therefore, equally possible that a DNA matching may cement a victim's identification of a suspect as her assailant as it is that it will contravene her most positive declarations of a particular suspect's guilt. The pending Chavez prosecution in California is a case in troubled point.

Rigoberto Chavez was accused of a most brutal kidnapping and rape of a San Mateo County woman in 1987. At a preliminary hearing on the charges the victim without so much as a flicker of a doubt identified Chavez as the culprit. But DNA matching of three semen samples recovered from the crime scene contradicted the victim's eye-witness identification.

The stage was then set, in the Chavez case, as it is already elsewhere, for a pitting of the direct evidence of an eyewitness against the circumstantial evidence of a scientific evaluation. Such a winner-take-all scuffle may be a first for DNA matching, but it is not at all a new phenomenon for science to be at odds with eyewitness assertions. As it happens, the classic illustration of such a clash of testimonial virtues occurred in the trials and tribulations of Adolph Beck, the case that was the corner-stone for the acceptance of fingerprinting in England almost a century ago. And so we have now come full circle. The lessons once learned in fingerprinting should not have to be relearned in the second coming of DNA matching, but apparently such is not to be the case.

Adolph Beck was twice confounded, once in 1896 by his conviction and sentence to seven years at hard labor for bilking numerous maiden ladies of their jewelry and again in 1904 by his conviction for a similar series of crimes. In both trials Becke's alleged victims trooped into court in droves and unquestioningly identified him as their swindler. Even two police officers queued up in further support of the victims' identifications. Beck

protested his innocence, but to no avail.

Beck spent five years in prison for his 1896 convictions and he was about to be shunted off to another lengthy prison stint for his 1904 convictions when the terrible truth became known. Beck had been mistaken for William Thomas, alias John Smith, who was a Beck look-alike. Both men were in their middle fifties, of portly carriage, grey-haired, mustachioed and monocled. These resemblances apparently provided the stimulus for the victims to state their most convincing and conclusive misidentifications of Beck, misidentifications they all instantly recanted when William Thomas was paraded before them.

When fingerprinting, then a nascent law enforcement tool, was conducted on the persons and objects which had been purloined in the Thomas' "Murphy game" trickery, Beck was fully exonerated. There followed such a public outrage as England had hardly ever encountered in past instances of legal injustices. Science, stolid police work and old friend luck had put the quietus to claims of eyewitness certainty, but not until Beck had suffered much.

Like fingerprinting, its forensic progenitor, DNA matching is not error free. But if properly validated protocols are steadfastly followed and well trained personnel are at the helm, the chances of error are negligible. The results of DNA matching, therefore, should be given greater credence in the legal arena than the markedly error-prone testimony of eyewitnesses. The law needs no Adolph Beck redux to add to its embarrassments.

Regardless of the fragility of eyewitness identifications, in rape cases and others, DNA matching can give law enforcement, at the investigative level, a decided uplift in rape cases. Serial rapists, such as was shown at the penalty phase of the Timothy Spencer trial, can be demonstrated to be at their malevolent work through DNA matching of biological samples from the individual and disparate rape victims. Law enforcement needs to know whether one rapist is on the prowl or more than one. For sentencing purposes too, the issue is singularly relevant.

Traditional forms of blood grouping tests are also inadequate to the tasks of gang rape investigations.

The mixed bodily fluids can be separated through their DNA content and identified to one rapist or to another, giving support to the victim's recollections or providing the necessary evidence where the victim was too traumatized to have a reliable memory of the events.

In many respects DNA matching in the legal order is tracking the path of its forebears among other scientific developments. What fingerprinting was to the justice system at the turn of the century, DNA matching is to the legal world in the 1980s.

Just as it is the law's pride to exhibit a briny irreverence in the face of scientific authority, so it is also the law's fame to embrace a scientific discovery of stature and proven merit.

Things did not fall apart when fingerprinting arrived on the legal scene a century ago. Nor will the center not hold with DNA matching having claimed its share of legal attention. Clearly there are scientific nodes yet to unwind in DNA technology and defense attorneys must be revivified in their adversarial tasks.

However, adversarial ardor, I have no doubt, can only have an annealing effect on the forensic legitimacy of DNA matching so as to dispel any portents of chaos in this second coming. It is only to criminals and others of that ilk that the dawning of DNA in the legal world should come as a warning.

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DNA ! Where Were You When I Needed You?

by Prof. Jarnes E. Starrs, senior editor

Budgets, budgets everywhere and not a dollar to spend on high tech scientific testing.

To what extent should a crime lab's budgetary constraints be permitted -- in law -- to explain away the state's failure to conduct costly but more exacting scientific tests to pinpoint, ever more definitively, a suspect as guilty or not? That was the question -- in its most succinct form -- before the Sixth Federal Circuit on the habeas corpus appeal of convicted rapist Gary Wayne Beam.

Beam had been convicted in a Michigan state court of various crimes stemming from the rape of a store clerk. The victim, who worked the midnight shift at a convenience store, testified that her assailant entered the store ostensibly to buy a Pepsi and, upon approaching the counter to make his purchase, he pulled out a gun and forced the woman to accompany him to a nearby wooded area where he raped her. The victim was then obliged to return with her attacker to the store where she was instructed to hand over all the money in the cash register, after which the rapist made his escape.

The police were immediately summoned and the victim assisted a detective in making a composite drawing of her assailant. She also looked through several hundred photographs provided by the police and identified a photo of Gary Beam as that of her attacker. So far so good, for the state. In a line up, the victim again identified the defendant as the perpetrator and, later still, in the courtroom, where she stolidly claimed that "there's no doubt in my mind" that Beam was the man. In the face of such a mountain of incriminating evidence of a most compelling eye-witness, what, if anything, might Beam bring to the fore to check it?

The defense asserted that Beam was the victim of misidentification, honest and sincere though it may have been. It was argued that there were powerful reasons for the jury to doubt that Beam was the miscreant.

Beam himself testified that, on the night of the rape, he was at the home of some friends, accompanied by his fiancee. All of those persons testified in support of Beam's alibi. Further, fingerprints found on an unopened can of Pepsi that the rapist had left in the woods did not match the defendant's prints. And although the victim testified that her assailant had no mustache, there was testimony that Beam was, at all relevant times, mustachioed.

Notwithstanding Beam's case for misidentification, he was convicted and his Michigan appeal went for nought. In the Federal Courts he raised constitutional claims. His principal argument centered on the prosecution's assertion at the state trial that Beam's blood type matched that of the rapist, as determined from semen samples obtained from the victim's vagina. The analysis of the seminal fluid revealed that the donor was a type O secretor. Beam was also type O secretor. The defense argued strenuously that the blood test results could not be construed as satisfactory evidence since the prosecution had at its disposal much more sophisticated testing procedures that could exclude all but 1% of the population as the rapist. The prosecution, it was said, was under a legal and constitutional obligation to make use of such tests.

A witness from the state police crime laboratory testified that the police lab was able to perform tests that could have substantially narrowed the number of potential donors of the recovered semen, but that such tests were not performed. It was said there was a "very severe budget crunch," coupled with serious understaffing so the "only the minimal amount of work is possible to do in any given case." On cross-examination, Beam's attorney alluded to the accuracy and specificity of "biochemical testing procedures and enzyme testing and the potential of such testing methods to greatly limit the number of possible suspects." In reply, the state's expert admitted that such procedures could eliminate a great many suspects, but that no such tests were run in the case at bar.

The prosecutor countered that it was not the state's burden to conduct every conceivable test in order to eliminate all potential suspects save the accused. Admitting that other tests could feasibly have been called to the task, it was the prosecutor's view that the defense could present such evidence on its own, if it was so het up about its importance. Beam's counsel discounted the existence of such a duty but did not object to the prosecutor's shifting the onus to him. The jury was instructed that "the law does not require a defendant to prove innocence or to produce evidence whatsoever."

Beam stated on this appeal that the failure of his counsel to object to the prosecution's statement regarding the burden of proof misled the jury and denied him the effective assistance of counsel. The Federal court looked at the matter differently.

The jury had been properly instructed as to the burden of proof, said the appeals court, and the

defense had made it known that Beam had no obligation to procure any serological evidence of his own. Any prejudice resulting from the prosecutor's remarks was, therefore, trivial, at best.

Circuit Judge Merritt, in dissent on this issue, was moved to protest that the prosecution's statements were demonstrably prejudicial. Noting that the prosecution's case was based on very weak scientific evidence, the dissent feared the jury might have been taken in by the prosecution's remarks shifting the burden of producing evidence to Beam.

Although DNA typing was not undertaken in this case, nor was it even addressed by the parties or the state or federal courts, still it is a lingering presence. The state of the fast developing science of DNA typing gives sufficient pause to reflect on the possible outcome of this case, which was a toss-up on the facts, if DNA testing had occurred.

.....*Beam v. Foltz*, 832 F.2d. 1401 (6th Cir. 1987).

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Signs of the Times: DNA Stars in Virginia Legal Firmament

by Prof. James E. Starrs, senior editor

There ought to be a Hall of Fame for law enforcement officers. If there were, Detective Joe Horgas of the Arlington County, Virginia police would be in the front and center rank of those first to be enrolled. Without a flicker of a doubt, but for the law enforcement perspicacity and pluck of Detective Horgas, Timothy Spencer would still be on the Virginia streets burglarizing, raping and murdering in the serial way that had become his signature.

Horgas was assigned to investigate the death of Susan Tucker, 44, who was found in bed, trussed up and nude, in her Arlington home after the 1987 Thanksgiving Day weekend. The condition of her body indicated that Mrs. Tucker had been dead for some few days.

A broken downstairs window evidenced a burglar's entry. The autopsy revealed that Mrs. Tucker had been raped or sodomized or both. Her husband was proved to have been out of the country preparing for a relocation of the family to Great Britain. There were no eyewitnesses to the crime or to any peepers, leerers or gawkers in the neighborhood of Mrs. Tucker's residence. Detective Horgas had a detective's Gordian knot to unwind.

Horgas catalogued the various features of the Tucker murder that seemed distinctive. The breaking and the entry, the private home, the lone woman, the tying up of the victim, the rape were all suggestive of a pattern that Horgas recalled from a number of unsolved burglary-rapes committed in Arlington County in 1983. But the 1983 crimes had, unaccountably, ceased late in January 1984.

The 1983 crimes had two additional elements: the offender had been masked and armed with a knife. Horgas decided to chance fate by tacking these ingredients onto the profile he was formulating of the Tucker murderer.

Checking and rechecking proceeded apace with police departments in other areas of Virginia. A Richmond rape-murder in early September 1987 had many of the features of the Tucker killing and the Richmond killer was still at large.

Horgas consulted the F.B.I.'s Violent Criminal Apprehension Program to press his inquiries nationwide, employing the profile that he thought best fit the Tucker murder. But all leads were futile dead ends until Horgas zeroed in on the dates that appeared most hauntingly telltale.

In January 1984, the burglary-rapes ended in Arlington County and in September 1987 they recommenced in Richmond, or so Horgas' best investigative speculations led him to surmise. Where was the culprit during that period? The F.B.I. had already informed Horgas that serial rapists do not spontaneously renounce their marauding ways. So his rapist must have been, somewhere and somehow, disabled from engaging in his favorite nefarious pastime.

Horgas honed in on the records of persons incarcerated in the Virginia state prison system during the three and a half years in question. And eventually but not inevitably, after the fashion of the tireless labors of the F.B.I., which revealed the fingerprint card of the murderer of Martin Luther King Jr., Horgas came upon a person who had been sentenced to prison after an Alexandria burglary conviction in late January 1984. And this confinement followed by scant days the cessation of the unknown burglar-rapist's depredations.

Further checking disclosed that this same convicted burglar had been paroled to a half-way house in Richmond just two weeks before the unsolved Richmond burglary-rape-murder had occurred. As further evidence that the trail was blazing hot, Horgas learned that the parolee in question had been on a weekend furlough from his Richmond half-way house both when the Richmond and the Arlington murders had been perpetrated.

The pattern seemed too compelling to be coincidental and sufficiently probative for the issuance of an Arlington bench warrant for the arrest of Timothy Spencer for burglary, a crime that Spencer had been convicted of committing six times before in his twenty-six years of life.

With the arrest of Spencer in Richmond by Horgas, the case against him as the Tucker murderer took more precise and persuasive shape, indeed by leaps and bounds. Spencer made statements incriminating to himself as he and Horgas travelled the one hundred miles from Richmond to Arlington (No "Christian burial speech" induced these disclosures.) Scientific analyses of hair, glass and semen pointed accusingly at Spencer. And the DNA typing evidence set at one in 135 million the chance that a black other than Spencer deposited of the biological samples at the Tucker crime scene.

The DNA typing became the sword and buckler of the prosecution's case.

On July 15, 1988, a day signed by Cancer but, more appropriately, within the dawning of the age of DNA typing, a jury of eight women and four men convicted Timothy Spencer of rape and capital murder in the killing of Susan Tucker.

On the next day the same jury, after a capital sentencing hearing at which DNA profiling was introduced to link Spencer to two Richmond rape-murders, recommended the death penalty. Trial judge Benjamin Kendrick set sentencing to follow the Richmond murder trials in September 1988.

Aside from the percipient investigation by Detective Horgas, what was single and distinctive about Spencer's murder trial? It was not the first trial in which DNA typing had been introduced into evidence. The *Pestinikas* case in Pennsylvania (see S.S.N. Vol. 11, No. 3) lays valid claim to being the first such trial. The widely publicized Florida rape prosecution of Tommie Lee Andrews was a second trial use of a DNA identification.

Spencer's trial might erroneously be thought, as most reporters recounted, to be the first capital murder usage of DNA typing. Au contraire! The first instance of that nature was the Putnam County, Florida rape-murder prosecution of Randall Scott Jones in March 1988 in which DNA "fingerprinting," as conducted by Cellmark Diagnostics, was instrumental in swaying the jury to convict, and, quite possibly, also to sentence Scott to death.

No, Spencer's trial was *sui generis* because it was the first trial of a serial rape-murderer in which DNA typing was introduced. It was also the first full scale criminal trial in Virginia in which a DNA identification had survived an admissibility challenge.

It was also remarkable for the ineptitude of the defense attorneys who, at the pre-trial admissibility hearing, ran the gamut of conceding the admissibility of DNA typing, then offering to stipulate to that concession, then withdrawing the stipulation and engaging in a paltry, half-hearted cross-examination of the prosecution's expert witnesses on DNA typing.

Indeed, at the trial itself defense counsel prefaced his cross-examination of the prosecution's chief expert, Dr. Michael Baird of Lifecodes, with the apology "I feel inadequate to the task of cross-examining you." His cross examination then and at the pre-trial hearing proved that his prefatory disclaimer was all too correct.

Defense counsel did not even advert to the small imperfections in the perfectionist veneer of Dr. Baird who had claimed membership in the American Society of Forensic Sciences when it is properly the American Academy of Forensic Sciences. Further Dr. Baird referred to publications in the *American Journal of Forensic Sciences*, when one assumes he misstated since there is today only a *Journal of Forensic Sciences*. Such little errors might crack the composure of even the most steely perfectionist, as any authentic trial advocate knows.

Although the prosecution lacked a single eyewitness tying Spencer to the Tucker murder scene, it had no dearth of circumstantial evidence. Hair samples from the murder site matched Spencer's hair in all microscopic characteristics. Glass found in the pocket of Spencer's jacket was traced by refractive index to the broken glass at the point of burglarious entry to the Tucker home. Three genetic markers in the semen from the scene of the crime matched those in Spencer's blood.

The victim's husband was conclusively excluded as a contributor since he is a non-secretor and he was in absentia to boot. A state serologist was allowed to state that only 13% of the Virginia black population had comparable genetic markers in their bodily fluids.

On cross-examination, the defense made lame references to one in eight persons sharing the same genetic markers as those of Spencer and the samples from the crime scene. The state's expert would not parry with defense counsel on this matter and responded, quite emphatically, that 13% was the figure she gave. And she did not waver from that figure or go off on defense counsel's tangents. The jury, on its part, gave no visible sign that the point of the defense cross, such as it was, was either understood by them or impressed them in Spencer's favor.

In the presence of this weighty circumstantial evidence, and a weak, relative-supported alibi on behalf of Spencer, the prosecution's presentation of its evidence of DNA profiling was almost superfluous. Certainly, in terms of other cases regularly tried, it was almost overkill.

But the DNA forensic juggernaut was not to be stymied.

At a pre-trial *Frye*-type admissibility hearing (out of juror earshot once Judge Kendrick realized that the veniremen who were inexplicably taking it all in should be unceremoniously excluded) the prosecution impeccably introduced the testimony of its three experts in support of DNA profiling.

Judge Kendrick attentively listened to and observed the proceedings, including the projection

of 35mm slides and overhead transparencies. At one point, the judge interrupted Dr. Baird to ask if the digestion of DNA by restriction enzymes and the migration of DNA fragments during gel electrophoresis were not, as he believed, one and the same process. Dr. Baird replied unsmilingly and patiently as if he were leading a blind man across a treacherous roadway.

During Dr. Baird's elaborating on the methodology of DNA typing, the prosecution made a particular point of establishing a firm statistical foundation for Dr. Baird's DNA identification of Spencer as the malefactor. An article entitled "Human Population Genetic Studies of Five Hypervariable DNA Loci" co-authored by four persons (including Dr. Baird) from Lifecodes was put into evidence without any objection from the defense. This article had not yet been published, nor had it even been accepted for publication at the time of the pre-trial hearing. The defense attorneys apparently were stargazing as these happenings transpired before them.

In support of Dr. Baird, both at pre-trial and the trial, the prosecution called Dr. Richard John Roberts, Assistant Director at Cold Spring Harbor Laboratory, the laboratory that, the prosecution was at pains to emphasize, was directed by the James Watson. As Dr. Roberts detailed his lengthy and stunning professional credentials, the spectators could visualize the Spencer comet tailing off into a conviction.

The cross-examination of Dr. Roberts was of the most random, disorganized and perfunctory sort, giving him every occasion to use facile word play to curry favor with the jury. The defense made a stab, but an errant one, at the population statistics underlying the probes used by Lifecodes. But the defense was just not up to the refinements of probing the statistical underpinnings of the Lifecodes methodology.

When the defense's turn at bat arrived, Spencer's conviction was all but a *fait accompli*. No experts appeared for the defense on DNA typing or on anything else for that matter. Spencer took the witness stand with a swagger and a stare and gave the jury less uneasiness about convicting him, which they did after five hours of deliberations.

The capital sentencing hearing, which took the better part of Saturday, July 16, gave Spencer his due: a very tawdry laundry list of a lifetime of violent crimes and kindred misfeasances. The jury's recommendation of death was received with impassivity by Spencer, whose only condescension to emotion was to sign the courtroom with his upwardly thrust middle finger as he departed. No astral sign was that, nor Marc Antony's *signe of Battaile*.

In a not uncharacteristic epilogue, the defense attorneys were reported as being uncertain whether to contest the DNA evidence received at the trial on the forthcoming automatic appeal of the conviction and sentence to the Virginia Supreme Court. Even if the issue is raised on appeal, there is no trial record to give credence to the challenge. So all the present signs are favorably disposed toward an affirmance on appeal.

One never knows, for interpreting the stars is a risky business.

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Common Myths Concerning DNA

MYTH:
The entire DNA sequence in every individual is different from every other individual (except monozgotes).
REALITY:
DNA is composed of about three billion base pairs, of which only one in a thousand differs from person to person.

MYTH:
Any biological sample from a human being can be DNA profiled.
REALITY:
DNA is found only in nucleated cells (eukaryotes). Thus none can be isolated from saliva (unless epithelial cells are present) or the epidermal layer of skin or semen (unless sperm or epithelial cells are present) or blood (unless white blood cells are present) or hair (unless the root is present).

MYTH:
So long as DNA can be isolated from a sample it can be profiled.
REALITY:
High molecular weight (determined by thousands (kilos) of base pairs) DNA is necessary, unless amplification by polymerase chain reaction is used.

MYTH:
The DNA in each nucleated cell in a particular human being is the same. Consequently, sperm cell DNA is the same as white blood cell DNA for the same person.
REALITY:
Differences do exist in the DNA in the various nucleated cells in the same person, but these differences are not sufficient to compromise current DNA typing methodologies.

MYTH:
The number and length of particular DNA fragments produced by restriction enzyme cleavage will be unique to each individual, even siblings.
REALITY:
The size and number of DNA fragments resulting from a specific restriction enzyme give no basis for a specific discrimination among individuals since individuals evidence a variety of restriction site locations.

DNA Has Weathered Its First Appellate Court Rack; Florida Takes the Prize

by Prof. James E. Starrs, senior editor

The nagging wait is over. The fretful days are done. Florida can exult. It is the first state to decide at the appellate court level on the admissibility of DNA profiling in the legal arena. And it decided, by a unanimous court, that DNA was properly admitted to corroborate the identification of Tommie Lee Andrews as an Orlando, Florida rapist. The six page opinion was authored by Judge Orfinger for the Fifth District Court of Appeal and was rendered on October 20, 1988.

In a no-nonsense, workmanlike and rather prosaic fashion, Judge Orfinger's opinion spelled out the factual underpinnings of the state's case against Andrews, the prosecution's DNA testimony and the relevant evidentiary rules in Florida warranting the acceptance of this DNA testimony.

The Andrews case has been a well-traded commodity for some time. It has reached levels of national prominence due to newspaper and even mini-series television interest since the housebreaking and rape from which it sprang occurred in Orlando on February 21, 1987.

The case against Andrews was all circumstantial and then entirely of a physical evidence character. Andrews was literally brought to book by scientific evidence and nothing else, or so the court's arraying of the facts would lead one to conclude.

Standard serological testing methods established that semen from an *O* secretor was found on fluid from the victim's vagina. Andrews is an *O* secretor. The victim is a type *O* non-secretor, but because the victim was savagely and wantonly assaulted and caused to bleed profusely, the state's serologist stated that the type *O* reading from the semen could have originated either from Andrews or the fluid blood of the exsanguinating victim.

Much more to Andrews' trial dismay, a fingerprint expert identified two latent crime scene prints as belonging to Andrews. These prints had been lifted from a screen removed by the intruder in securing entry to the victim's residence.

Thus far the state's case against Andrews was compelling but not overwhelming, and certainly not conclusive. To add to the certainty of Andrews guilt the state presented expert testimony from two experts from Lifecodes (one of three commercial enterprises functioning in the DNA genetic testing field) that the DNA from the offender's sperm obtained from the rape victim "matched" Andrews' DNA within "a reasonable degree of scientific certainty." The defense objected to this testimony, arguing, in the main, that the methods and procedures in performing the matching were flawed. The scientific acceptance of DNA was not the particular focus of the defense's opposition, but rather the propriety of the handling of the technique on this occasion. The trial court rejected the defense's effort, unsupported as it was by any expert testimony, to blockade DNA from the jury and the Florida Fifth District Court of Appeal has now upheld this determination and Andrews' convictions.

The Controlling Legal Standard

The first order of business for the Florida reviewing court was to decide what legal standard controls the admissibility of such innovative scientific techniques and principles as DNA. In view of the somewhat unsettled state of the law as propounded by the Florida Supreme Court, the state's highest appellate body, Judge Orfinger decided that either the *Frye* or general acceptance rule, on the one hand, or the relevancy/reliability rule of Florida's Evidence Rule 702, on the other, might be the prevailing guide.

After a thorough review of the Florida decisional law, it was concluded that DNA, to be admissible, must be shown to be helpful to the factfinder (read relevant here) and must have certain "indicia of reliability" in addition, which need not go so far as to equate with the more stringent general acceptance mandated by *Frye*. A gratuitous passing footnote made reference to the fact that even under the general acceptance test, DNA would pass muster.

"Indicia of Reliability"

The indicia of reliability of DNA profiling, as elucidated by Judge Orfinger, were:

1. The well-received scientific state of the art of DNA analysis as opposed to its being a new, novel and experimental exposition. "DNA testing has been utilized for approximately ten years" and is performed in a number of laboratories around the world, said the court.
2. The DNA test is used not exclusively in a forensic setting but has many other applications in the diagnosis, treatment and study of genetically inherited diseases.
3. A specialized scientific literature exists on DNA profiling, all of which supports its scientific validity.
4. The frequency of erroneous results is infinitesimally minute or non-existent.
5. Controls are utilized at every step of the

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process to keep errors to the barest minima.

6. The statistical data base from which the likelihood of a match is drawn, although based on 710 randomly selected samples by Lifecodes, was not challenged by any defense expert testimony.

The Defense Tactics

Running through Judge Orfinger's opinion are frequent references to the strategies of Andrews' defense counsel in its counterthrust to the DNA profiling evidence. No defense expert testified or was even proffered for that purpose. The defense aimed its arrows, exclusively through cross-examination of the prosecution's experts (two from Lifecodes and one from M.I.T), at the laboratory methods, particularly contesting the electrophoretic separation technique (based on purported inadequacies in the gel matrix) and the statistical framework for the ultimate finding of identity.

Witness Bias

Unlike his judicial confreres, or consoeurs, in Michigan and California, Judge Orfinger was not perturbed by the claimed "built-in bias" of the expert witnesses for the state. This supposed lack of objectivity was said, by the defense, to arise from the fact that the Lifecodes personnel who testified had constructed "their reputations and careers" on the commercial success of their DNA print technique. Judge Orfinger, in a footnote response, refused to be periphrastic on the subject. "Neither *Frye* nor our evidence code require impartiality" of expert witnesses, nor did the contention otherwise have substantial merit where other non-forensic usages for DNA profiling exist.

Science and the Court

Fortuitously, Judge Orfinger kept his discussion of the science of DNA profiling to the barest acceptable minimum of a paragraph or two. However, even then, he did not seem to be cognizant that the Lifecodes DNA process of single locus probes, which was at the core of this controversy, was fundamentally distinguishable from the Cellmark Diagnostics multi-locus probes, otherwise known, in a somewhat infelicitous phrasing, as DNA "fingerprinting." Indeed, in the first sentence of his opinion Judge Orfinger refers to "genetic fingerprinting evidence by which strands of coding" in the DNA molecule are identified. Seemingly the difference essential for DNA profiling purposes, and other uses as well, between the coded portions (exons or extrons) and the uncoded segments (introns) of the human genome had not pierced this judicial veil.

All in all Judge Orfinger's opinion is well-deserving of "bouquets and ribbon'd wreaths." But it is as yet too soon to say with assurance that the ship of DNA is "anchor'd safe and sound," either in Florida, where the state's Supreme Court may have the final say, or elsewhere where the issue is yet to be tested.

.....*Andrews v. State*, 1988 Fla.App. Lexis 4645.

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Defense Attorneys Muck About; Defenses Going Every Which Way

The scurrying and the foraging is frantic, frenzied and decidedly madcap. No long-barred attic is too remote. No bottom drawer is too abandoned or ill-remembered. No cluttered attic is too forbidding. Defense attorneys are at their wits end in rummaging about for legal bulwarks against the muscled and molecular might of the DNA juggernaught. In proof we abstract two judicial decisions, one from Texas and the other from Ohio.

In Texas:

For all we know -- and we know very little from the spare bones of the Houston, Texas appeals court decision -- Henry Lee Bethune had good reason to be worried. His anxiety presumably stemmed from being a suspect in some unidentified crime or crimes where DNA "fingerprinting" was to be on center stage.

Before Bethune would consent to walk through the proscenium arch to his trial, he wanted to be certain he had drawn a judge who was "board certified" as a criminal law specialist knowledgeable about DNA "fingerprinting."

Judge Azios of the Harris County District Court just did not meet the measure of Bethune's high expectations. So he moved to recuse Judge Azios and to replace him with a "board certified criminal specialist." Nothing was said about the qualifications Bethune thought would be required of a judge suited for the intricacies of his DNA-laden criminal trial. Nor did the Houston reviewing court dare to delve into them.

Bethune's motion was dismissed for lack of procedural merit. He had made no effort to demonstrate that a DNA neophyte as a judge would necessarily be one whose "impartiality would be called into question." The sole ground for such a recusal motion would be to challenge the trial judge's impartiality. Incompetence on the subject of DNA, even if presumed, does not equate with a bias against the accused.

Certainly if Bethune could frustrate his trial reckoning until such time as a DNA-skilled judge came on the scene, it would be a 90 degree day in the Arctic when he could be tried at all.

The stalling effort was unique, but its novelty did not carry the day.

.....*Bethune v. Azios, Judge*, 1988 Tex. App., Lexis 2491 (Tex. App., 1st Div.)

Whereas in Ohio:

Apparently Robert Biddings had a penchant for rape. It is precisely in such cases that DNA profiling has found a certain and well-received niche in the legal system.

Biddings tried to stymie a court order for the taking of his blood to compare its nuclear DNA component to that found on various of his alleged rape victims. His principal objection was that giving blood would constitute a serious violation of his religious scruples. The Ohio appeals court for Franklin County was not impressed.

Biddings was shown to be an affiliate in a rather inactive way with the Jehovah's Witnesses. The court was troubled by the apparent insincerity of his religious connection and by the lack of proof that the religious tenets of the Jehovah's Witnesses spoke against the giving of blood,under these circumstances. Biddings' failure to testify at all at a hearing on the issuance of the search warrant for his blood was eyed as showing something less than proof positive that his religious convictions were truly at issue. He just did not bear his burden of proving that he had "an honestly held religious view that prohibited the taking of blood samples from him." But Biddings had more cards up his sleeve.

The court order should not be implemented, he said, because less intrusive means exist to get the same biological information. To support this assertion, Biddings sought to introduce a brochure from Cellmark Diagnostics of Germantown, Maryland, the company scheduled to do the DNA testing once his blood was drawn. This brochure, so it was advanced by Biddings, proved that hair and nucleated cells from bodily tissue and secretions other than blood could do the testing job as well. And obtaining exemplars of them was concededly less invasive of his bodily integrity.

The Cellmark brochure, standing alone and unsupported by sworn testimony, was deemed inadequate for Biddings' purposes. He might have made a stronger argument if this "unsworn and inconclusive brochure" had been supported by other more concrete evidence. The appeals court was not about to take judicial notice of the contents and the accuracy of the Cellmark brochure, nor was that matter even in issue.

The defense's delaying tactic went for nought. Even if it had prevailed, the testing would have gone forward with other tissues or specimens from Biddings. His claim just bought him time. With what goal in mind, no one then or now would conjecture.

.....*State v. Biddings*, 1988 Ohio App. Lexis 4179 (Franklin County).

Statement At A Press Conference At The
National Press Club on September 17, 1987

Celebrating the English Connection and the American Experience

Remarks by

James E. Starrs
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This celebration of the advent of DNA fingerprinting in the U.S. could not have been better timed nor more opportune. Today can be truly called the day when we celebrate the English connection in two, quite interrelated, respects. The recent barrage of media coverage from all sides has made it patent to all and sundry that today we commemorate the 200th anniversary of the signing of the United States Constitution in Philadelphia. THis document, we now realize, had its roots not merely in our struggle for independence from Great Britain but also in any number of fundamental legal guarantees long extant in English law and practice. In confirmation of this juridical connection, one need point only to the ancestry of our due process of law mandate in the "per legem terre" injunction in Chapter 39 of the Magan Carta.

Just as our Constitution has distinct blood lines reaching back into English law, so DNA analysis is beholden to the pioneering work of the Nobel prize winning British-American team of Francis Crick and James Watson. And today we welcome Professor Alec Jeffreys of the University of Leicester to our shores and we commend his efforts to make DNA analysis an integral feature of forensic science in the courts of this land.

From my perspective as a lawyer and a forensic scientist, it can legitimately be said, without fear of hyperbole, that the influence our Constitution has wielded over the years in the sphere of government, DNA analysis will in due course come to reflect in the legal order. Let me illustrate why this prediction prognostication is not simply airy professorial humbug.

Assume a woman has been raped and murdered and the police investigation is proceeding energetically. The victim's clothing reveals traces of what proves to be a mixed stain combining vaginal and seminal fluids. Testing according to the ABO blood group system gives assurance of the presence of the A blood group in the stain. Whether the A antigen reaction derived from the semen or the vaginal fluid portion of the stain in unknown and, at present, scientifically unknowable. Indeed, the A reaction could have resulted form bacterial contamination of the stain. The plot thickens when it is discovered that the victim is of the A blood group herself and is a secretor to boot.

Once a suspect is developed, as the police euphemism has it, the inquiry is directed to whether he was the donor of the semen discovered on the victim's clothes. The time has now arrived for science to make the all-important, critical connection between the suspect and the crime scene evidence.

The contemporary process of doing so is convoluted and tortured in its performance and uncertain in its results. Two essential findings are sought. First, is the suspect one of a sizeable number who secret their blood group into bodily fluids other than blood, such as semen and saliva? second, if so, is the ABO blood group of the suspect the same as that identified in the mixed stain from the victim's clothing?

Obviously the first question can best be resolved after analysis of a sample of the suspect's semen. Here, however, constitutional protections come into focus. Both due process and search and seizure concerns are implicated in seeking to obtain semen samples from an unwilling suspect. In default of semen, a saliva sample will be the next best and constitutionally acceptable alternative. But saliva is not semen and scientific conclusions drawn from saliva, because of aberrant reactions and the like, are not necessarily transferable to semen.

In an effort to unsnarl this enigma, attention is directed to obtaining a blood sample from the accused. But on this matter fourth amendment constraints will ask troubling questions concerning whether the blood sample is to be obtained intravenously or by the less intrusive subcutaneous pricking of the tip of a finger. Is there sufficient probable cause to do either and was a judicially issued search warrant employed in the enterprise?

Assuming that all these constitutional hurdles are surmounted and the suspect's blood sample proves that he is blood group A, as was the victim and the crime scene stain, how persuasively will this data finger the accused as the culprit ? It is undisputed and indisputable that under such a scenario the accused is only minimally and only speculatively pinpointed as the miscreant. That's the abysmal state of the art that we are constrained to live with today. How might DNA analysis both untwist the current scientific snarl and provide more meaningful and positive results?

DNA analysis, when full blown after being perfected by validation studies and by time and cost containments as well as otherwise, will enable us to determine and to distinguish the DNA types within the mixed seminal-vaginal stain in my very real-life scenario, which is something no current methodology can lay claim to do with any degree of assurance. It will also enable us to bypass constitutionally problematic procedures involving forcible blood letting or compelled semen sampling. Hairs, which would be within the ken of identifying characteristics constantly exposed to public view and, consequently, removed form the shoals of the Fourth Amendment, would be secured form the recalcitrant suspect and analyzed for their DNA component. Then the DNA type gleaned from this hair analysis would be compared to that found in the crime scene stain and, mirabile dictu, either the accused is our man or he is not within a hair's breath of absolute certainty.

And those almost miraculous prospects are not polyanna nor are they all we can expect of DNA analysis in the near future. I predict that DNA analysis will put an end to microscopic hair comparisons as a means of connecting a suspect to a crime scene. And putting the quietus to such baleful microscopic comparisons the hazy testimony resulting from it will lift a heavy burden of dispute and even rancor form the courts and the forensic sciences. No longer will there be niggling and flyspecking over the number of microscopically identifiable features and the gamesmanship of statistically narrowing the field of possible donors to the accused.

Moreover, I confidently predict the fingerprint files now proliferating in this age of the computer will be supplemented, if not displaced, by DNA files of equal size and of greater practical moment. Criminals can were gloves to mask the friction ridge characteristics of the palmar surfaces of their hands but they cannot so readily hide the details of their DNA identifier. What fingerprinting was to law enforcement at the end of the 19th century, DNA analysis will be at the turn of this century and then some.

As a lawyer qua forensic scientist I foresee an illustrious future for DNA analysis in the administration of criminal justice, among other legal uses, as for example, in paternity testing. However, for reasons of scientific polity and out of long acquaintance with the evidentiary rules for the admissibility of scientific evidence in the courts of law, I would urge caution and prudence in the rush to make DNA analysis a fixture in the courts. With Alfred Lord Tennyson, I would maintain that science must move "but slowly, slowly, creeping in from point to point."

If the blandishments of notoriety, profit and urgency are conscientiously eschewed, I would foresee that DNA analysis will be the milestone in the legal firmament that the United States Constitution was to the development of the United States as a whole. Just as the Constitution, once signed had to be ratified and tested over time, so DNA analysis must be similarly solidified. Then the English connection that both the Constitution and DNA analysis represent will be quickened and fortified by its American experience.

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DNA IN THE JOURNALS

THE GOOD, THE BAD AND THE MALADROIT

by Professor James E. Starrs

As a topic of wide-ranging public appeal of late, DNA knows no equal - that is, if the journals and reports, both popular, scientific and legal are to be credited. And that question is the focus of this review. Can the proliferation of recitals, typically overarching in their praise of DNA on the printed page be relied upon as accurate and responsible commentaries on the forensic status and applications of DNA technology? In brief, is the subject of the forensic uses of DNA receiving its just due through the written word?

Out of an urge to be merciful, little need be said of the coverage of DNA by the fourth estate. DNA is regularly described in the press as a genetic marker. And so it is, indeed the primordial one. But then, in an excess of zeal to be first, the press has proclaimed that DNA's courtroom appearance constitutes the first time that genetic markers have come into courtroom play. Not so! Genetic markers, in the form of blood group typing, have been performing quite handsomely in the courts long before the arrival of the new kid, DNA, on the genetic block.

The press has even reported the hyperbole of some of the proponents of the forensic applications of DNA as incontrovertible matters of proven fact. In a July 18, 1988 report entitled "Justice Seen Through a Microscope," Insight reporter Daniel Kagan quotes Dr. Robert Shaler to the effect that "(t)he criminal justice system has never been able to conclusively identify a person" until the advent of DNA profiling. Not so! Fingerprinting has been performing that function in a agreeable fashion for nigh on to a century. And it will not be ousted by DNA profiling.

But everyone knows that the press coverage of just about everything is tainted by biases, subliminal or otherwise, as well as the mad dash to condense and to meet copyroom deadlines. But what is the performance rating of publications that are not beset by the vagaries that lead the popular press to give a wide berth to accuracy. At this more rarefied and sophisticated level of publishing endeavor, there have been articles and reports that span the horizon and the good, to the bad, to the maladroit.

But first the good news.

The Good Way

As one could readily surmise, reports on DNA typing for legal purposes that have emanated from the leading forensic laboratories in the English speaking world have been uniformly superb in all respects. In England, where the Jeffreys' multi-locus probes are under intensive scientific investigation by the Home Office Forensic Science Service's Central Research Establishment (HOCRE), the publications of HOCRE have represented a very balanced view of DNA's forensic applications.

In HOCRE's 1986 Annual Report, for example, a blind trial is reported to have revealed "that vasectomized individuals do not produce sufficient somatic cells in their semen to give a DNA profile." That preliminary findings should motivate the collection and DNA testing a vast array of physical evidence from the crime scene. The DNA that may not be found in the semen may well be located in the blood, hair or bodily tissues discovered at the crime scene. It is simply the application of the well-worn apothegm that admonishes not to put all your eggs in one basket (in this instance, semen).

A number of HOCRE's Reports on DNA fingerprinting have been republished in more accessible scientific journals. Dr. Peter Gill's articles are particularly impartial, comprehensive, exacting and enlightening. Two of them are worth special mention, viz., "An evaluation of DNA fingerprinting for forensic purposes" (Electrophoresis 8:38-44, 1987) and "A new method for sex determination of the donor of forensic samples using a recombinant DNA probe." (Electrophoresis 8:35-38, 1987).

On the more western front, the F.B.I. Laboratory, at its Quantico, Virginia facility, is presently undertaking what can fairly and without pejorative intent be denominated a crash program on DNA profiling.

But two published documents elucidating the G.B.I.'s efforts and DNA profiling in general have appeared this year in Crime Laboratory Digest. These are notable contributions and should be

essential materials for all persons who wish to be informed on the background and developments in DNA profiling for forensic purposes.

The first, "An Introduction to the Methods of DNA Analysis Under Investigation in the F.B.I. Laboratory," was published under the co-authorship of Budowle, Deadman, Murch and Baechtel in the January 1988 issue (15:8-21). Aside from its needful but elementary description of the structure of the DNA molecule, the article summarizes the highlights in DNA technology that are common to all probes. It gives particular attention to the effects of point mutations on the possibility of mismatches and to the method of amplifying (duplicating) DNA by polymerase chain reaction (PCR) when the crime scene sample is either too degraded or contains DNA in an insufficient molecular weight for profiling. The two plus pages devoted to "Validity Considerations" could well be a point by point list for prosecution and defense in preparing or challenging an expert witness on this subject. It includes vital aspects of quality control within the laboratory and peer review of scientific findings, through publication in scientific journals or otherwise. Issues relating to chain of custody are addressed in connection with recommendations to evaluate the effect of "storage time" on "polymorphic patterns."

The "Validity Considerations" pinpointed by the F.B.I. should be read side by side with the recommended "Standards for Parentage Testing Laboratories" of the American Association of Blood Banks (AABB). Such a comparison is necessitated by the fact that the F.B.I.'s grasp is much broader than the AABB's reach.

The AABB, thus far at least, has limited objective. Only paternity cases (crime scene contaminated samples are excluded.) and those where a single locus rather than a multi-locus probe of the Jeffreys' variety, familiarly known as "DNA fingerprinting," are within the ambit of the AABB's standards. In spite of these announced limitations, the AABB's proposed standards are more grist for the courtroom mill where either Frye-type admissibility issues are at stake or a laboratory's compliance with an accepted Protocol for DNA testing is under close scrutiny.

On the omnipresent issue of insuring quality control within the laboratory, the AABB's proposals speak volumes. The following are among the salient elements in that schema: (although renumbered for present purposes)

1. A method shall be available to assure complete endonuclease digestion of DNA for testing.
2. A human DNA control of known size shall be used on each electrophoretic run.
3. Autoradiographs or membranes shall be read independently by two or more individuals.
4. Confirmatory testing by an independent laboratory shall be possible for all DNA loci.

A second F.B.I. report appeared in July 1988 as Supplement No. 1 to Volume 15 of Crime Lab Digest. It is titled "DNA Implementation." There are a number of strictly laboratory oriented articles in this issue on the cost of initiating a DNA profiling program, cautionary charges on the use and disposal of radioactive materials and an outline of the course content for a training program in DNA analysis.

A bibliography organizes the relevant literature into discrete categories, which, although sometimes overlapping, provide a means of easy entry to the readings that one might reference on any point in the vast spectrum of DNA's forensic concerns.

Although disclaiming any pretense at being all encompassing, the bibliography does omit Rankin and Wink's article "Method and Application of DNA fingerprinting: A Guide for the Non-Scientist" (1987 Crim. L. R. 105-110). For lack of a better laymen's guide, the Rankin and Wink opus, although more prosaic than the other listed references, deserves at least coordinate billing.

The feature article in the Supplement, "A Primer on the Methods Used in the Typing of DNA" by Baechtel, is what the title states it to be and more. The author candidly admits that the band patterns in the autorad generated by a multi-locus probe are "more complicated in interpret than for single locus probes." That factor may well give momentum to the computer aided reading of an autorad.

With typical but commendable F.B.I. overscrupulosity, Baechtel finds it to be at present merely "conceivable" that a conclusive matching of specimens could result from using "only a single multi-locus probe." If, however, eleven bands, say, were to match in single multi-locus probe and the sharing of bands among persons is one in five, then the resulting statistical conclusions make a match

somewhat better than "conceivable."

As to the matter of sample size, something of a bete noir for DNA typing thus far, certainly for multi-locus probes, the article give hope of a more promising future. It is said that recognizable patterns have been found in controlled laboratory samples of semen stains as infinitesimal as "1/50 of the volume of one drop from an eye dropper." Recognizing that crime scene samples are to controlled laboratory tests what an iguana is to an elephant, still the laboratory tests provide a glimmer of hope that the size of the sample will not be a major impediment to a broad-based use of DNA profiling.

So much for the good news. Now for the bad.

THE BAD WAY

The Congress of the United States deserved better than it got. Maybe not perfection or even what passes for perfection in the capital city but better than it got in the discussion of DNA typing that appears in the May 1988 Special Report of the Office of Technology Assessment (O.T.A.) entitled Criminal Justice: New Technologies and the Constitution. The report's two page summary of the state of l'affaire DNA is flawed both on the scientific and the legal aspects of the subject. The scientific transgressions are on quite rudimentary matters and can be catalogued as follows:

1. The very first sentence of O.T.A.'s report on DNA typing states that "DNA...is...found in every cell of the body." At the expense of being cast aside as a picker of nits, it needs to be said that only nucleated cells contain DNA. Thus mature red blood cells are not within the ken of DNA typing. That this is not a trifling oversight by the report's draftman is made manifest by an equally fundamental error of forensic significance that occurs in the report's discussion of the Tommie Lee Andrews rape trial in Orlando, Florida in 1988.

2. The report states "semen found in the ...rape victim was a 'perfect match' to that of the accused." It is not scientifically possible to match semen samples, even supposing the most implausible even that the accused either voluntarily or coercively gave a semen sample for testing. A court order for a semen specimen would be outrageously intrusive and, as such, a violation of either or both the Fourth Amendment or the Due Process Clause of the Fourteenth Amendment. What one surmises the report intended was to say the sperm from the semen collected from the victim was matched to the blood of the accused, Tommie Lee Andrews. There is a difference in more than degree in these two statements of the same ultimate proposition.

3. The O.T.A. reporter writes "the nucleotides are sequenced in a different pattern in each individual." That statement obscures and muddies the scientific fact that of the three billion or so base pairs that comprise human genomic DNA, a staggeringly large percentage, some have said 999 out of every 1000 bps, are shared commonly among human beings. It is only the smallest fraction of sequencing of the necleotides that enables DNA typing to discriminate one individual from another.

4. The report refers to the English experience in the use of DNA fingerprinting in the following horrid fashion: "In England, genetic typing is accepted as conclusive evidence." The authority for this declaration of fact is not the HOCRE reports nor articles in Nature and other scientific and legal journals, but a one page review of DNA in Newsweek! Worse yet, nothing in support of the report appears within or between the lines of the Newsweek item referenced.

No evidence of any kind is ever considered to be conclusive in a court of law. Not even a fingerprint identification state to be a positive one is conclusive. The jury, in its ultimate wisdom, may reject that finding out of hand, so to say. Indeed, an instruction to the jury that it has such authority is the norm in most courts.

Moreover, it is not "genetic typing" in all its permutations that the O.T.A. reporter has in mind but one species of such typing called DNA profiling. If genetic typing is conclusive evidence, then proof that an accused is blood type "O" is also conclusive. But, conclusive of what?

So much for the bad news. Now for the really bad news.

The Maladroit Way

On the representation that no one has as yet "adequately" adumbrated the subject of DNA analysis "in any legal journal," White and Greenwood co-authored "DNA Fingerprinting and the Law" the March 1988 issue of The Modern Law Review. Greenwood is footnoted as a member of the Law as well as the Biological Sciences Departments of the University of Dundee. White's professional affiliation is not reported.

The article gives exclusive attention to the Jeffreys multilocus probes and, in that connection, addresses British cas law in which the Jeffreys' technology has been received. The article contains any number of curious comments; one might describe them as wondrous locutionary gawkers. For example the reader is informed that only in "some rapes" is trace evidence left at the crime scene, whereas it is found in "many murders." The authors provide no support for those gratuitous assertions, nor could they as a matter of fact. At another point, the reader is left bedazzled by the remarkable assertion that "the most obvious use of determining parenthood is in case of disputed paternity."

On the subject of DNA fingerprinting, the article is woefully deficient both one the scientific mooring for DNA analysis and on its legal applications. Some murkiness in expression is tolerable, especially in scientific papers, but it is unabashed nonsense to describe as "difficulty" in paternity cases the fact that DNA profiling "has no application to fiances and fiancees, and can only identify spouse if they have children."

The article is rife with sticky wickets, demanding clarification, in its description of the technology of DNA fingerprinting. Since the authors, in their very first footnote, express their appreciation to Dr. Jeffreys for his review of "an earlier draft," I asked Cellmark Diagnostics to rechannel the crooked ways of this article. The comments of Bruce M. Lehr, Market.

Minisatellites:

In striving to elucidate the minisatellites which are central to an understanding of the Jeffreys' technology, White and Greenwood say: "Each individual minisatellite is present in only a proportion of the population, but there are so many that each person carries numbers of them."

In reply:

The term 'minisatellite' refers to regions of the DNA where a short 'core' sequence of nucleotide bases is repeated many times in succession (so-called tandem repeats). The number of times the core sequence is repeated can vary. However, the number of repeats does not have to vary. The specific 'minisatellites' detected by the Jeffreys' DNA probes do vary in the number of times the core is repeated. At given genetic loci (defined sites on chromosomes), differences among individuals may be detected due to this variation in the number of times the core sequence is repeated.

Restriction Enzymes:

"To take a DNA fingerprint, one extracts the DNA...and treats it with enzymes that chop it all up into short pieces: all, that is, except for the minisatellites."

In reply:

In the DNA Fingerprinting process, the restriction enzyme is specifically chosen so that it will not cut the repeating core sequence one wishes to detect with the Jeffreys probes.

Electrophoresis:

After an electrophoretic run, say White and Greenwood, "the DNA is invisible, as one cannot see directly how far the various pieces have travelled."

In reply:

The result of electrophoresis is a continuous smear of DNA fragments, of varying size, that is not interpretable, even if visualized through the application of a visualizing reagent like ethidium bromide, which fluoresces under UV light.

Hybridization

"When a solution of the probe is poured over a gel, the probe DNA attaches itself to any minisatellite DNA."

In reply:

Before hybridization with a probe, the double-stranded fragment of DNA must be denatured by severing the hydrogen bonds. Cellmark does this denaturing in the course of electrophoresis by an alkaline wash. The DNA fragments in the electrophoretic gel are than transferred to a nylon membrane by a process called Southern blotting. The hybridization process takes place on the nylon membrane, not in the gel. The Jeffreys DNA probes are then used on the nylon membrane to detect fragments that contain the repeating core sequences.

Autorad:

"If one lays an X-ray plate in the gel...one obtains a picture of the distribution of the minisatellite DNA."

In reply:

The X-ray film is brought into contact with the hybridized fragments on the nylon membrane, not put directly into the gel, which at this point in the process is valueless for further identification objectives.

Data Banking of DNA

"It would be technically feasible...to build up a database parallel to an ordinary fingerprint library...potentially covering the entire population and largely without its knowledge."

In reply:

One point that is often misunderstood in the talk about data banking of DNA requires mention. DNA fingerprints depend upon the DNA probe and restriction enzyme used to generate them. Different probe/enzyme combinations will result in different DNA fingerprints.

Therefore, if multiple jurisdictions want to generate compatible DNA databases to facilitate the identification of suspects from unknown samples, they must compare results from the same probe/enzyme combinations in their databases. For example, a DNA profile from a suspect generated using the Jeffreys probe MS31 and the restriction enzyme Hinf I must be compared to a similar profile in a data base generated using MS31 and Hinf I.

The supplement Mr. Lehr's last critique of White and Greenwood, it would seem unrealistic, and otherwise Don Quite-like, to propose that such a vast database could be established "largely" without the knowledge of the entire population of collecting hair, blood, etc from an entire, unsuspecting population goes beyond the realm of credulity.

L'Envoi:

The object of this exercise in the good, the bad and the maladroit ways in the DNA profiling literature was to alert the scientific, legal and law enforcement communities to the imperative need to be discriminating in identifying the DNA literature of genuine value, especially when the literature is emerging in such plentitude. One should be as discriminating in this identification process, one might add, as DNA profiling is in its turn. The results of a failure to be discriminating in either instance can be cataclysmic, both scientifically and legally.

ONE IN A SERIES OF FORENSIC BIBLIOGRAPHIES
PROVIDED BY

SCIENTIFIC SLEUTHING REVIEW

THE FORENSIC USES OF DNA ANALYSIS
A Bibliographic Benchmark

by Prof. James E. Starrs, senior editor

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A back to school refresher or first-time initiation into DNA and RNA - a solid and comprehensible work by a leading scholar and forensic scientist.

2. Hall, S.S., *Invisible Frontiers*, Boston: Atlantic Monthly Press, 1987.

The cloning of the gene for insulin and its being synthesized is told with the drama of scientific history and the clash of commercial exploitation.

3. Judson, H.F., *The Eighth Day of Creation*, New York: Touchstone, 1980.

A compendious and utterly readable, but lengthy, volume on DNA, its discovery and the fact that "the force" is now known to us all.

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3. Kelly, K.F., Rankin, J.J., & Wink, R.C., "Method and Applications of DNA Fingerprinting: A Guide for the Non-Scientist", *Criminal L. Rev.*, pp. 105 - 110, 1987.

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4. Lomax, I.S., "DNA Fingerprints - A Revolution in Forensic Science", *Law Society's Gazette*, pp. 12 - 13, 23 Apr. 1986.

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New York: The Foundation Press, 1986, 3d. Ed.

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7. Werrett, "DNA Fingerprinting", *Inter. Crim. Pol. Rev.*,
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3. Jeffreys, D.J., Wilson, V., and Thein, S.L., "Individual Specific 'Fingerprints' of Human DNA", *Nature*, vol. 316: 76 80, 4 July 1985.

Discusses various Jeffreys' probes and analyzes the statistical basis for identification through DNA analysis.

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1. Di Lonardo, A.M., Darlu, P., Baur, M., Orrego,C., and King, M-C, "Human Genetics and Human Rights", *Am. J. For. Med. Path.*, vol. 5: 339 346, 1984.

The Argentinean kidnaping and killing of young and old alike and the unparented children left behind detailed and diagrammed.

2. Diamond, J.M., "Abducted Orphans Identified by Grand-Paternity Testing", *Nature*, vol. 327: 552 - 553, 18 June 1987.

A reference, by and large, to H.L.A. testing to establish grand-parentage but DNA mentioned as particularly germane when one grandparent is

unavailable for testing.

3. Michaud, S.G., "Identifying Argentina's 'Disappeared' ", *New York Times Magazine*, 12/27/87, p. 18.

The search for the roots of Argentina's surviving children of pogrommed parents is elucidated a la the New York Times fashion.

RAPE:

1. Giusti, A., Baird, M., Pasquale, S., Balazs, I., and Glassberg, J., "Application of Deoxyribonucleic Acid (DNA) Polymorphisms to the Analysis of DNA Recovered from Sperm", *J. For. Sci.*, vol. 31 (2): 409 - 417, 1986.

A Lifecodes approach to DNA analysis of sperm which sings its praises to the loftiest heights based on a study of blood and semen samples donated by 120 volunteers.

2. Kanter, E., Baird, M., Shaler, R., and Balazs, I., "Analysis of Restriction Fragment Length Polymorphisms in Deoxyribonucleic Acid (DNA) Recovered from Dried Bloodstains", *J. For. Sci.*, vol. 31 (2): 403 - 408, 1986.

Methods and materials employed in Lifecodes analysis of DNA in blood explicated. Aged (three year old) bloodstains said to be effectively identified. Environmental factors may be a failing which more study will resolve. The number and frequency of the alleles detected is said to be crucial to an identification.

TIME OF DEATH DETERMINATIONS:

Perry, W.L., Bass, W.M., Riggsby, W.S., and Sirotkin, K., "The Autodegradation of Deoxyribonucleic Acid (DNA) in Human Rib Bone and Its Relationship to the Time Interval Since Death", *J. For. Sci.*, vol. 33 (1): 144 - 153, 1988.

A provisional, conditional and otherwise highly tentative report on the results of DNA analysis of 7 rib bone samples, without disease, donated by the University of Tennessee for study of the degradation of DNA over time. "Some evidence" existed that the time of degradation is similar among unrelated individuals but bones other than rib bones may not be in agreement. Many issues and hypotheses remain to be tested, but the results of this very limited study argue mightily for further experimental analysis.

In the Courts:

ENGLAND:

The unique and almost Ripley-like tracking of the dual rape murderer, Colin Pitchford, in the English Midlands which culminated in his guilty plea and sentence to life imprisonment. Jeffreys and his DNA analysis were prominent throughout.
Schmitz, A., "Murder on Black Pad", *Hippocrates*, Jan/Feb. 1988, pp. 48 - 58. The latest and most thorough discussion is in Wambaugh, J., *The Blooding*, New York: Wm. Morrow & Co, 1989.

U.S.A.:

1. The Pestinikas trial (Scranton, Pa.):

The death of Joseph Kly of malnutrition in 1984 brought Walter and Helen Pestinikas to trial for homicide in Scranton, Pa. Although the prosecutor's requested DNA analysis was admitted into evidence at the trial, it was of benefit to the defense, not the prosecution. Consequently, a full-scale testing of DNA's evidentiary value was postponed to another day. *Scientific Sleuthing Newsletter*, vol. 11(3): 1, 4-6, 1987.

2. The death penalty for Anthony Apanovitch (Ohio):

The dissenting judge maintained, unpersuasively to the majority, that the state should be compelled to utilize DNA testing prior to the imposition of the death penalty. *State v. Apanovitch*, 514 N.E.2d 394, 406 (Ohio 1987).

3. The paternity of Donald Lane Flora (Utah):

Although H.L.A. testing was used and, as submitted, found unacceptable on appeal, DNA was mentioned, tangentially, in connection with a discussion of the Standards for Parentage Testing of the American Association of Blood Banks. *Kofford v. Flora*, 744 P.2d 1343 (Utah 1987).

4. Chromosomal-variant Analysis (Maryland):

DNA analysis is mentioned in a neutral fashion, au courant, in deciding that chromosomal variant testing does not meet the test of admissibility in Maryland. *Cobey v. State*, 533 A.2d 944 (Md. 1987).

5. Legal Admissibility:

a. The first appellate court admissibility

determination results in approval of DNA in a Florida rape trial. *Andrews v. State*, 533 So. 2d. 851 (Fla. 11/10/88).

b. The first written and published opinion from a trial court admitted DNA profiling after a lengthy "Frye" type hearing. *People v. Wesley (and Bailey)*, 533 N.Y.S. 2d 643 (Co.Ct.Albany, N.Y. 1988).

6. Tangential Aspects of DNA Analysis:

a. due process denied in refusing defense requested continuance to interview prosecution's DNA experts. *Hill v. State*, 535 So. 2d 354 (Fla., 12/22/88).

b. religious scruples against blood testing insufficient to force prosecution to extract hair instead of blood for DNA testing. No proof hair analysis is equal to blood analysis. *State v. Biddings*, 1988 Ohio App. Lexis 4179 (Ohio App., 10/14/88).

c. Motion to recuse judge for lack of DNA knowledge rejected. *Bethune v. Azios*, 1988 Tex. App. Lexis 2491 (Tex. App. 10/6/88).

7. Paternity:

DNA testimony admitted in New York paternity dispute since it is a "blood genetic marker test" under the N.Y. Domestic Relations Law. No "Frye" or other admissibility hearing necessary. *Matter of Adoption of Baby Girl S*, 532 N.Y.S. 2d 634 (Surr. Ct., N.Y. Cty, N.Y. 1988).

THIS ARTICLE FIRST APPEARED IN *SCIENTIFIC SLEUTHING NEWSLETTER* VOLUME 12, NO. 3.

A First Look at the First DNA Data Banking Legislation
Colorado Enacts Mandatory Genetic Testing Statute

At 9:10 A.M. on May 29, 1988, Governor Romer of Colorado made history for Colorado and the nation when he put his signature to House Bill #1081. The legislation makes Colorado the first state to require genetic testing of all sexual assault offenders who are released on parole on or after that date. Colorado thus becomes the only DNA data banking state, following the lead of the Seattle (King Count) ordinance (#8453) approved in March of this year, but not to be implemented until after October 1, 1988 or later, if certain civil libertarian safeguards are not then in place.

Concededly, California has, since 1983, had state-wide legislation imposing very similar testing requirements to these newly minted in Denver. The difference between them inheres in the nature of the tests to be conducted. The California law (Penal Code 290.2) speaks quite explicitly of "analysis and categorizing into blood groupings." That kind of genetic typing is not the focus of the Colorado statute, which is much more broad-based; it refers to determining "the genetic markers" in the blood of the prospective parolee, which, in theory and in plain English, will include DNA as well as other blood group type genetic markers. The Seattle ordinance, for its part, directs that DNA typing be performed on all convicted felony sex offenders, whether to be paroled or not. Only the Seattle ordinance puts DNA into the legislative lights, in so many words.

In California and Colorado the trigger for genetic testing, of whatever sort, is the parole of a sex offender. This seems a very niggardly approach to the public safety needs of the people - for DNA or other genetic profiling - but is a beginning, withal. The costs of the implementation of the Colorado statute are directed to be borne by the "victims assistance and law enforcement" appropriations, with an advisory amount of $18,000 appropriated for the current fiscal year.

Even though it is a very compact one section enactment, one still might inquire why the Colorado statute refers to "chemical testing" and, indeed, what that phrasing signified to the legislative mind. Further, the statute requires the giving of blood and saliva (for secretor status determinations). We are left to wonder why hair, urine (for epithelial cells) etc. are not either specified or included under broad-brush terminology like biological specimens. We are also in the dark as to how the essential computerization of the program will operate. But the statute does limit access to the data generated "to any law enforcement agency." That may salve some civil liberties anxieties.

THIS ARTICLE FIRST APPEARED IN *SCIENTIFIC SLEUTHING NEWSLETTER*, VOLUME 12, NO 3.

DNA as a prosecutor's sword; Not an accused's shield

She said he did and he said he didn't. That is often the way forcible rape trials bottom out, especially where the accused claims to have been elsewhere at the time of the occurrence.

But in the Alabama forcible gang rape trial of John Andrew Parker the physical evidence also gave some support to the defendant's claim of non-involvement. The hair and semen samples taken from the victim did not match Parker's. Parker, at the time of the gang rape, had an active case of herpes but the victim did not contract the disease, even though she said Parker had been the second of the five persons who raped her.

In this state of the evidential affairs, Parker alleged that his conviction and sentence to life imprisonment without the possibility of parole should be overturned. the alabama reviewing court was not convinced that the evidence was insufficient as a matter of law to show Parker's guilt.

It was all a matter of "probabilities and not absolutes" according to the appellate court. His herpes was not certain to be transmitted nor was his hair or semen necessarily to be found deposited on the victim.

Forcible rape requires non-consensual sexual union, all of which can be proved without proof of emission. In the last analysis, the jury is charged with the resolution of disputed facts and, on this score, the court did not deign to upset the jury's finding of guilt on the disputed facts.

DNA analysis was not reported to have been conducted in Parker's gang rape prosecution by the value of DNA typing in gang rape prosecutions should not be overlooked or overestimated. DNA typing, unlike traditional forms of antigenic testing, can discriminate the separate and distinct DNA molecules of the various persons whose seminal fluid is found as a mixed stain taken form a rape victim. Thus a DNA typing in that instance which matches an accused's DNA is very instrumental in proving the prosecution's case, at least that sexual congress occurred.

But the flip side of this scientific coin does not similarly boost the defense's not guilty claim. A failure to match the accused's DNA to that in the mixed vaginal sample does not make the unaccused's innocence even minimally plausible (unless the intercourse). As the Alabama appellate court rightly said it is. *Parker v. State*, 516 So. Dc. 859 (Ala. Ct. App. 1987).

Senator SIMON. Thank you very, very much.
Dr. Peterson.

STATEMENT OF JOSEPH L. PETERSON

Dr. PETERSON. Good morning. My research has focused on the uses and effects of scientific evidence on police and judicial fact-finding, plus the quality and fairness of forensic science services offered the criminal justice system.

Despite notable improvements in recent years, the potential of forensic science has not been realized because of limited resources. DNA typing holds tremendous promise to discriminate persons through their biological fluids and tissue and to associate persons with their crimes with virtual certainty. DNA's actual impact will rest on our judicial system's resolve to provide the needed scientific resources and to insist on the highest professional and judicial standards.

Between 1980 and 1986, my colleagues and I undertook two national research projects aimed at determining the rates of usage and the impact of forensic science evidence on the investigation and prosecution of serious crimes.

We reviewed several thousand cases from the files of crime laboratories, police agencies, and prosecutors' offices. We also interviewed prosecutors and defense attorneys and surveyed jurors about the credibility and comprehensibility of different types of evidence and testimony.

About two-thirds of crime laboratory caseloads today are devoted to drug and driving under the influence of alcohol cases. Limited resources prevent laboratories from examining a substantial quantity of evidence recovered from the scenes of personal and property crimes.

At the court level, we found forensic laboratory reports in about one-quarter of charged cases. Results of laboratory testing demonstrated a connection between the defendant and the crime a small percentage of the time.

For example, blood stain evidence associated murder defendants with their crimes in only about 15 percent of cases. Semen evidence associated accused rapists with their victims about 10 percent of the time, and hair evidence associated defendants in less than 5 percent of murders and rapes. Laboratory results which excluded defendants were even more rare. DNA typing holds great promise for improving these rates of association and disassociation.

We found solution rates of offenses with scientifically analyzed evidence to be, on average, about three times greater than cases without such evidence. The breakthroughs in recent years in automated fingerprint identification systems and now with DNA should enable police to make substantial improvements in their rates of solution.

In particular, DNA technology holds the promise of making a substantial contribution to the investigation of stranger-to-stranger sexual assaults. The key here will be, one, developing technology capable of deriving DNA from the semen, blood, and hair left by the unknown assailant; two, building population frequency data;

and, three, which may be the most controversial, creating files containing DNA information on prior violent or sexual offenders.

About three-quarters of charged offenses result in a conviction, usually on a plea to the original charge. While individual cases with scientific evidence resulted in higher rates of conviction, the fraction of cases with scientific evidence linking the accused to the crime was so small in relation to the total body of cases being prosecuted that forensic science evidence overall did not prove to be a major predictor of case outcome.

Consequently, for scientific evidence to have greater impact on our system of justice, resources are required to extend these scientific protocols to a greater proportion of cases proceeding through our court system.

Scientific experts testify in a small percentage of trials, which is a further indication of resource limitations. Trial attorneys agree, however, that when they do testify, experts have substantial impact. Juries expect scientific evidence to be presented in trials, particularly in rape cases. They perceive, they understand it, and they are troubled when it is absent.

Jurors rank forensic experts the most persuasive of all witnesses who testify before them. About a quarter of the jurors reported that without the forensic evidence, they would have changed their verdicts, usually from guilty to not guilty.

The overwhelming majority of forensic evidence presented at trials is introduced by the prosecution. Budgetary limitations usually prevent defense counsel, particularly those defending indigent defendants, from hiring their own experts to review the State's scientific evidence. As a result, defense counsel seldom engage in a meaningful review of the government laboratories' test results or interpretations of the evidence.

The presence of forensic laboratory reports significantly increased the likelihood of incarceration in attempted murder and robbery convictions. Forensic laboratory reports were also associated with longer prison sentences, especially in the crimes of attempted murder, rape, and robbery.

Evidently, prosecutors are less inclined to bargain with defendants where forensic evidence is present and judges are more willing to sentence convicted defendants to maximum terms.

My principal recommendations fall into five basic areas.

1. RESOURCES

Although forensic science has made tremendous strides in the past two decades, the profession still lacks the resources to fully capitalize on physical evidence. Implementation of DNA analysis will require added funds. Resources will be needed to continue the research, to develop and refine laboratory techniques, to continue validation and persistence studies, and to establish data bases.

Forensic science university programs must be supported to engage in research and train new examiners in these methods. Policy studies are needed to determine the optimal location of laboratories with forensic DNA testing capabilities. Funds will also be required to hire scientific staff at Federal, State, and local levels.

2. EDUCATION AND TRAINING

DNA technology presents a new challenge to the forensic science field, and to meet that challenge requires highly trained scientific staff. It is clear, too, that the successful utilization of DNA will require knowledgeable judges, prosecutors, and defense attorneys. Training programs and workshops will be necessary to familiarize these legal personnel with the basics of the technique, as well as its proper application and interpretation.

3. QUALITY ASSURANCE

Rigorous quality assurance programs must be in place to ensure that DNA and other forensic test results meet the highest scientific standards. The crime lab profession has made substantial strides in the past 10 years in improving their quality assurance programs, and these programs should be expanded.

The American Society of Crime Laboratory Directors offers a voluntary crime laboratory accreditation program to ensure the integrity of physical evidence and the final work product. This program should be extended.

Crime laboratory proficiency testing gives laboratories the opportunity to test their proficiencies in examining different types of evidence. Many States and locales have now initiated their own internal testing procedures. So, both internal and external proficiency testing programs need to be supported and expanded.

Several subdisciplines within the forensic sciences offer certification programs which recognize individual examiners. In the very near future, criminalistics professionals will initiate their own certification process to review the education and training, experience, and proficiencies of individuals.

No routine procedures exist at present for the testing and evaluation of routine scientific methods in use in forensic laboratories. In the DNA area, the FBI laboratory is assuming a leadership role in forming such standards, but similar initiatives are needed in other areas of forensic examination.

Ethical codes call upon experts, regardless of their employer, to express both the strengths and weaknesses of their examinations and not to give opinions which exceed the limits of their data. Such problems are exacerbated when scientists are required to practice under severe caseload pressures.

4. ANTICIPATED IMPACT

DNA typing should have impact on many crimes of violence, as well as in many other cases of victim identification. One of the areas of greatest impact will be in the investigation and prosecution of stranger-to-stranger sexual assaults.

With the creation of DNA data bases, investigators should be more successful in solving crimes and linking together offenses committed by the same assailant. Equally or more important is DNA's potential to exculpate wrongly accused defendants. DNA should evolve into one of forensic science's most discriminating and reliable techniques for distinguishing the innocent from the guilty.

5. EQUITY

Last, although the great majority of scientific evidence in the criminal courts is offered today by the prosecution, the examination and interpretation of scientific evidence must be an objective, nonpartisan undertaking.

The examinations and results of analyses performed in Government laboratories must also be available for review and verification by the defense. A spirit of openness should prevail in crime laboratories to satisfy both scientific and judicial standards.

Given the scientific sophistication, probative value, and the expense of the DNA typing process, it is important that our judicial system provides the resources and the mechanisms for the defense to gain full access to this technology.

Thank you.

[The prepared statement of Dr. Peterson follows:]

FORENSIC SCIENCE, DNA TYPING AND THE CRIMINAL JUSTICE SYSTEM

Statement to the United States Senate
Committee on the Judiciary, Subcommittee on the Constitution

March 15, 1989

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Introduction and Background

Our criminal justice system has become increasingly reliant on scientific methods of criminal investigation and modes of proof. Scientific evidence is considered intrinsically more reliable than other forms of evidence, given its physical nature and the precision of measurements performed on it by impartial, forensic scientists. In the wake of rising crime rates, landmark judicial decisions curbing questionable police interrogation practices, pressures to upgrade and professionalize the police and courts, and the presence of Federal monies to finance justice system improvements, the number of crime laboratories more than tripled in the past two decades (Peterson, 1985).

However, given the loosely confederated structure of law enforcement agencies and forensic laboratories in our nation, the quality and impact of these scientific advances have not been uniform. Despite notable steady improvement in recent years, the potential of forensic science has not been realized due principally to chronic underfunding. My research has focused on the uses and effects of scientific evidence on police and judicial fact-finding, plus the quality and fairness of forensic science services offered the criminal justice system. In large measure, the contribution of the forensic sciences has been, and will continue to be, a function of the resources our judicial system is willing to offer this most important area.

Most of our research was completed prior to the introduction of DNA typing. However, using data collected in these earlier studies as a baseline, I hope to identify areas where the newly developing DNA typing technology will have its greatest impact, those which will have to be carefully monitored, plus programs adopted by the profession in recent years to assess the quality of personnel and the accuracy of test results.

The anticipated impact of DNA typing is based on its power to discriminate persons through their biological fluids and tissue and on its potential to associate persons with their crimes with virtual certainty. While anecdotal data indicate DNA

typing has had a dramatic impact in individual cases, its impact on a system-wide basis will not be known until the technology has been used more widely and the effects of the DNA results can be isolated from other factors influencing case outcome.

This paper will not directly address the scientific or theoretical basis of DNA typing, problems relating to the proprietary nature of probes, issues concerning DNA's admissibility into courts of law, or questions of privacy and confidentiality associated with the construction of computerized DNA data banks. These are all very important questions, but I will leave them to my distinguished colleagues on this panel.

Method of Approach

Between 1980 and 1986, my colleagues and I undertook two national research projects aimed at determining rates of usage and the impact of forensic science evidence on the investigation and prosecution of serious crimes (Peterson et al, 1984; Peterson et al., 1987). The research was based in six cities - Peoria and Chicago, Illinois; Kansas City, Missouri; Oakland, California; and Litchfield and New Haven, Connecticut. Sites were selected by their range in population, geographical location, volume of caseflow, and resources devoted to the collection and analysis of physical evidence.

We reviewed several thousand case files which were selected at random from the files of crime laboratories, police agencies and prosecutor's offices and recorded the frequency with which forensic evidence was examined and its impact on case outcome. In addition, we interviewed prosecutors and defense attorneys in the six locales to determine their perceptions of forensic evidence, as compared with other types of evidence. We presented hypothetical cases to prosecutors in Chicago to obtain their views on the relative impact of eyewitnesses, confessions, tangible and forensic evidence on case outcome. Finally, we surveyed approximately 300 jurors following their return of verdicts in felony trials and asked them about the credibility and comprehensibility of different types of evidence and testimony.

Forensic Evidence in the Judicial Process

The findings of this research may be summarized as follows:

Rates of Usage and Laboratory Results

The rates of usage of scientific evidence in personal and property crimes did not increase appreciably over the five-year period covered by this research (1975-1981), largely as a result of continually rising drug caseloads in the laboratories. About two-thirds of crime laboratory caseloads nationwide are devoted

to drug and driving under the influence of alcohol cases. Limited resources prevent laboratories from examining a substantial quantity of evidence recovered from the scenes of crimes in a comprehensive and timely fashion.

At the court level, we found forensic laboratory reports in about one-quarter of charged cases, a value that varied as a function of offense type. Five types of scientific evidence dominated: drugs, fingerprints, firearms, blood and bloodstains, and semen. Results of laboratory testing demonstrated a definite or probable connection between the defendant and the crime a small percentage of the time, which is principally a function of the quality of evidence and standards (samples of known origin) collected, the sensitivity of the scientific method employed, and the technical skill of the scientist.

Given the potential of DNA to link criminal offenders to their victims via biological evidence like bloodstains, semen and hair, it is useful to review these results of (pre-DNA) laboratory testing. Our study found blood and bloodstain reports in roughly a fourth of murder prosecutions with test results associating defendants with their crimes in only about 15% of cases. Semen evidence was examined in more than 40% of rapes, but associated the assailant with his victim only about 10% of the time. Hair evidence was reported in about 10% of murders and 25% of rapes, but associated murder and rape defendants to their crimes in only 2% and 5% of cases, respectively. Laboratory results which excluded defendants were even more rare.

DNA typing holds great promise for improving the rates of association and dissociation. Research presently underway is establishing the reliability of procedures, and should define the conditions (e.g., quantity and condition of the sample, or environmental factors) which may limit the information that may be derived from crime scene samples. Just as important will be the training of police and crime scene technicians in the proper recovery and preservation of samples from suspects, victims and scenes of crimes.

Arrest and Clearance

We began by seeing whether scientific evidence had an appreciable effect on the clearance rates of burglaries, robberies, and aggravated assaults. After controlling for other types of factors, we found solution rates of offenses with scientifically-analyzed evidence to be, on average, about three times greater than cases without such evidence. Scientific evidence seemed to have its greatest impact in those cases where the chances of solution were smallest - those without suspects immediately named and placed.

The ability of crime laboratories to help investigators solve

crimes is limited by resources police departments allocate to the processing of scenes of crimes as well as resources within laboratories to speed the turn-around of results to investigators. Detectives must recognize, preserve and collect evidence from scenes and standards from suspects and not view physical evidence as a "last resort" or simply as a way to promote good public relations.

In particular, I think DNA technology holds the promise of making a substantial contribution to the investigation of stranger-to-stranger sexual assaults. The key here will be 1) developing technology capable of deriving DNA results from the semen, blood or hair left by the unknown assailant; 2) building population frequency data; and 3), which is the most controversial, creating files containing DNA information on prior violent/sex offenders. Sound policies must be formulated to define the nature and form of data to be included in such files, and adequate safeguards established to control access to and protect the confidentiality of such files.

Charging

Scientific evidence has minimal impact on a prosecutor's charging decisions, save for its corroborative value. Limited resources and case backlogs usually prevent laboratories from supplying results to prosecutors prior to the time charging decisions are made. The exception is in drug cases where the laboratory analysis of the suspected controlled substance must be available for the case to proceed.

Plea Bargaining and Trials

The overwhelming majority (90 to 95% on average) of charged cases are resolved through pleas. The research literature indicates, and our data confirm, that cases with stronger evidence are more likely to go to trial (Jacoby et al., 1982). Prosecutors are less inclined to charge or sentence bargain with the defendant in such cases. A confession, on the other hand, usually leads to a guilty plea. From a forensic evidence standpoint, prosecutors noted that the absence or presence of weak forensic evidence would likely lead to a case being resolved through a plea. One might expect, therefore, that the introduction of DNA evidence would tend to push more cases to trial, particularly where the prosecutor refuses to bargain or where the defense wishes to challenge the scientific evidence before a jury.

In our sample of charged cases, about three-quarters resulted in a conviction, usually on a plea to the original charge. Generally speaking, conviction rates of cases with scientific evidence were not significantly higher than cases without. We did find rates of conviction were generally higher when forensic

evidence associated the defendant with the crime, but in only two locations were there a sufficient volume of associative laboratory findings to yield statistically significant differences. This is another important finding that relates to the resource problem - in my judgment forensic evidence had minimal impact at conviction/nonconviction stage because of 1) the low percentage of cases in which forensic evidence was ever analyzed and 2) the even smaller percentage of cases in which the analysis actually linked the offender to the crime. DNA is certainly responsive to this latter problem by providing the means to link a defendant with his crime with virtual certainty.

Our statistical analysis found the most important evidence variables predicting case outcome were: 1) defendant admissions/confessions, and 2) tangible evidence (e.g., stolen property, and articles of clothing linking the defendant to the crime). Forensic evidence had a significant main effect in predicting the outcomes of murders and burglaries. Where, for example, bloodstain evidence associated the murder defendant with the crime, conviction rates were significantly higher. In rapes, we also found an interesting result: conviction rates were significantly lower when the defendant denied committing the crime and a laboratory report was absent. Semen evidence connecting the defendant to the victim was associated with higher conviction rates, whereas hair evidence proved inconsequential.

Interviews with prosecutors revealed they were very concerned about cases where scientific evidence was either absent or weakly associated the defendant with the crime. They believed such cases were problematic, particularly where a jury expects such evidence to be presented.

Jury Trials, Expert Testimony and Juror Comprehension

Scientific experts testify in a small percentage of trials (from only 3% of trials in Chicago, to 12% in Kansas City, to 23% in Peoria), which is a further indication of resource problems. Laboratory managers and prosecutors prefer that scientists be called to testify sparingly since this reduces the time available to process evidence. Trial attorneys agree that when they do testify, experts have substantial impact. Juries expect scientific evidence to be presented in trials and are concerned when it is absent. Our surveys of jurors indicate they perceive they understand scientific evidence as well as or better than other evidence. Jurors rank forensic experts the most persuasive of all witnesses who testify before them. About a quarter of the jurors reported that without the forensic evidence, they would have changed their verdicts (usually from guilty to not guilty). As juror understanding of the forensic evidence presented by the prosecution improved, the likelihood of conviction increased.

Particularly in rape cases involving biological evidence did jurors consider the forensic evidence crucial. I think this bodes well for the enhanced discriminatory power of DNA typing in sexual assault cases where the issue is not one of consent, but of the positive identification of the perpetrator.

Defense Challenges

The overwhelming majority of forensic evidence presented at trials is introduced by the prosecution. Although defense counsel may attempt to have physical evidence excluded during pretrial evidentiary hearings, such efforts are rarely successful. Once the evidence is admitted and experts are permitted to testify, defense counsel seldom mount a rigorous challenge to the evidence. In fact, defense counsel commonly try not to draw attention to the laboratory test results, either by stipulating to the test results and/or by not cross-examining the expert.

Budgetary limitations usually prevent defense counsel from having their own experts review the state's scientific evidence. As a result, defense counsel seldom engage in a meaningful review of, or mount a significant challenge to, the government laboratories' test results or interpretations of the evidence. Since defense counsel are usually ill-equipped to undertake such a review, this places an even greater burden on the state to insure that proper quality assurance procedures are followed and that examiners take the role of objective and impartial fact-finders in their presentation of scientific results to the court.

Sentencing

In our sample of felony prosecutions, the majority of convicted felons were sentenced to prison (upwards of 70 to 80 percent, depending upon the offense category). Although the defendant's prior criminal record overwhelmed most other factors in the decision to incarcerate, the presence of forensic laboratory reports significantly increased the likelihood of incarceration in attempted murder and robbery convictions. Forensic laboratory reports were also associated with longer prison sentences, especially in the crimes of attempted murder, rape and robbery. What explains this?

We theorize that forensic evidence gives the judge added confidence the defendant is unquestionably guilty and is deserving of the maximum allowable sentence. More importantly, the forensic evidence gives the prosecutor a stronger bargaining position where he/she will be less willing to negotiate the charge or the sentence, and more inclined to push for the maximum term.

Discussion

My principal comments and recommendations may be categorized into five basic areas: laboratory resource needs, education and training requirements (for both scientists and lawyers), quality assurance programs, the anticipated impact of DNA typing on case solution and adjudication, and questions of equity between the state and the defense.

Resources

The lay person often holds an exaggerated view of the role forensic science plays in today's criminal justice system. Although forensic science has made tremendous strides in the past two decades, the profession is still grossly underfunded. Consequently, only a fraction of potential evidence is recovered from scenes of crimes, only a portion of that evidence is ever analyzed, and only a small percentage of analyzed cases benefit from experts testifying.

Our studies have indicated that crime laboratories typically receive less than 1% of their parent (police) agency's budget. This budgetary reality limits the number of cases which can be accommodated, the detail with which evidence is examined, and the speed with which results are returned to users; accordingly it restricts the impact of scientific evidence on the legal process.

The resources that will be needed to undertake DNA analyses are not trivial and added monies are required. Funds will be needed to continue the research to develop and refine laboratory techniques, to continue validation and persistence studies, and to establish data bases. Operating funds will be required at the federal, state and local levels to procure necessary laboratory resources, to hire scientific staff and train them, and to contract (where necessary) with private firms. Research is also needed to establish uniform methods of analysis and interpretation. Policy studies will be needed to determine the optimal placement and configuration of laboratories with forensic DNA testing capabilities.

Outside the forensic science field, resources will be required to provide needed training to law enforcement and judicial personnel. This is an absolutely critical stage which will be discussed more below.

Education and Training

The sine qua non of a successful forensic science operation is competent scientific staff. Given the special status accorded expert witnesses by our legal system and the weight given to their testimony, forensic scientists must possess the very best education and training. DNA technology presents a new challenge

to the forensic science field and to meet that challenge requires highly trained personnel.

It is clear, too, that the successful utilization of DNA technology will require knowledgeable judges, prosecutors and defense attorneys. Training programs and workshops will be necessary to familiarize these legal personnel with the basics of the technique, as well as its proper application and interpretation.

Quality Assurance

Quality should not be compromised in forensic science practice. Rigorous quality assurance programs must be in place to insure that DNA and other forensic test results meet the highest scientific standards. While research in the 1970s indicated laboratories were experiencing serious difficulties with certain types of evidence (Peterson et al, 1978), crime labs have made substantial strides since then in improving their quality assurance programs. For example:

o The American Society of Crime Laboratory Directors (ASCLD) offers a voluntary crime laboratory accreditation program to insure the integrity of physical evidence and the final work product. More than sixty laboratories nationwide have now been accredited under this program. ASCLD has also adopted a set of management guidelines (ASCLD Guidelines, 1987) which obligates laboratory managers to safeguard the "integrity and objectives of the profession and to ensure faith of the public in the quality of its practice" and to "provide their professional staff with the means necessary to maintain their professional and scientific skills and insist that they do so".

o First begun fifteen years ago under a grant from the Department of Justice, a crime laboratory proficiency testing program continues (now on a private, fee for service basis) which gives laboratories the opportunity to test their proficiencies (Lucas et al., 1985) in examining different types of evidence.

o Several subdisciplines within the forensic sciences offer certification programs which recognize examiners who possess the necessary qualifications to practice their specialties. In the very near future, criminalistics professionals will initiate their own voluntary certification process to review the education and training, experience and proficiencies of individuals.

o National and regional professional societies have adopted codes of ethics (e.g., see AAFS (1986) and CAC (1985)) which require its members to be competent, objective and truthful with respect to their qualifications, examinations and testimony. Forensic professionals rank the problem of incompetency as a foremost ethical/professional concern. They also agree that

scientists who fail to express both the strengths and weaknesses and who give opinions which exceed the limits of their data present a serious problem. These problems are exacerbated when scientists are required to practice under severe caseload pressures (Peterson and Murdock, forthcoming).

o Although no procedures exist at present for the regular testing and evaluation of scientific methods of analysis in regular use in the forensic science field, there is recognition by many that such procedures are necessary and desirable.

Anticipated Impact

The impact of forensic science evidence may be measured by the assistance it offers legal decision makers in coming to swift, correct and fair judgments.

DNA typing should have impact on many crimes of violence where biological evidence is present. Data collected thus far by private firms (Buls, 1988; Shaler, 1988) indicate high rates of conviction of defendants linked to their crimes through DNA "fingerprinting". We note, however, that in murder cases, where conviction rates are already very high, significantly higher rates are unlikely. The impact of DNA will likely be felt most strongly in the investigation and prosecution of sexual assaults. At present, laboratories are only moderately successful in linking assailants to their victims using conventional laboratory techniques, and DNA procedures should yield far more definitive information from semen and hair. With the creation of DNA data bases, investigators should be more successful in solving crimes and linking together offenses committed by the same assailant.

Although more difficult to measure, but perhaps of even greater importance, is DNA's potential to exculpate wrongly accused defendants. DNA therefore, like fingerprints, should evolve as one of forensic science's most discriminating and reliable techniques for distinguishing the innocent from the guilty.

Equity

At present, forensic science evidence is used principally as a tool of the police and prosecution. It is not surprising that the prosecution is most excited about the potential of DNA since it is they who have the burden of proof in criminal cases (Johnson, 1988; Michaud, 1988; Moss, 1988; Toufexis, 1988). However, for our adversary system to function properly, there must be a balance between the prosecution and defense. With respect to scientific evidence, the defense must be afforded the opportunity to review, verify and, if need be, to challenge the scientific results and interpretations offered by the government. Given the sophistication and expense of the DNA

typing process it is important that our judicial system provides a mechanism for the defendant to gain full access to this technology.

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Senator SIMON. I thank you very, very much.

So that we have for the record the very, very practical application of this, will either one of the witnesses give me an example of how DNA identification might be applied in a crime of murder, a crime of rape, whatever?

Professor STARRS. Well, in the case, for example, of what is called colloquially a gang rape where the question is raised as to whether a particular individual in the gang did actually sexually assault the woman, we can today—unlike prior methods of identification, we can identify from the mixed sample obtained from the woman, from the victim—we can identify who it was among these many people who actually raped her and who didn't. That is a very important breakthrough over existing technology.

Dr. PETERSON. In crimes of violence, as you well know, bloodstain evidence is created, and oftentimes blood is shed and transferred from victim to defendant. For many years, forensic scientists have attempted to link defendants to scenes of crimes and victims of crimes using blood evidence, as well as other forms of trace evidence such as hair.

DNA presents, again, the potential to link unequivocally a particular defendant to a particular case, not only with bloodstain evidence, but as I mentioned before, with hair evidence, which presently has not been able to yield that sort of definitive finding that is needed to form a basis and a conclusion of responsibility.

Senator SIMON. Do fingernail, semen, hair, and blood samples all show that same DNA pattern for Dr. Peterson, for Professor Starrs, for Paul Simon, for any individual?

Professor STARRS. The DNA that we are looking at, which is the uncoded regions, is the same in all the nucleated cells in the body. There would be obvious differences with respect to the coded regions because it performs different functions.

The DNA that is found, for example, in the nucleus of sperm has a different function from the DNA found in the roots of hair, but that is the coded region. We are only concerned with the uncoded, and that is uniformly the same throughout the entire body.

Senator SIMON. This is just a minor point, but the term DNA fingerprinting somehow doesn't adequately convey what we are doing. DNA profiling is maybe a little better. I will let you and the academic field come up with a good phrase.

Professor STARRS. Well, DNA identification is probably the optimum statement because we are striving for certainty of identification as to who the individual was or whether the individual was not involved in a crime.

DNA fingerprinting was a term of art chosen by Dr. Alec Jeffreys, who developed a system, a technology for identification in England, and he utilized the term DNA fingerprinting.

I prefer, because of the difficulties in interpreting this to lay people and distinguishing it from true fingerprinting—I prefer to call it either profiling or identification.

Senator SIMON. Now, how do we get people to give samples for DNA identification?

Professor STARRS. Well, Joe.

Dr. PETERSON. Samples of evidence may be present at scenes of crimes which are recovered, but what is needed are standard sam-

ples of known origin taken from victims, suspects, other parties who may have shed blood or hair or semen in a particular case.

So, through appropriate investigative and judicial procedures, samples of your blood or my blood or hair may be obtained from us, if necessary, through court order so that we may compare your blood with the blood left at the scene of the crime to see if it is indeed the same, or if the bloodstain on the cuff of your shirt is the same as the victim's blood.

And so it is this comparison process that takes place that enables us to say that you, again, have an association with that crime.

Senator SIMON. But that is where you have a known suspect. When I entered the Army, I was fingerprinted. In different spots in your life, you will go through fingerprint identification, and then if there is a crime committed and there is a fingerprint, you can go back to a central spot and determine who may have committed the crime.

When you go into the Army, when you enter a prison, when you go into a series of situations, are we headed toward that that DNA profile being put on record automatically? How do we get this catalog?

Professor STARRS. We are very much headed in the direction by State legislation at this time of DNA data profiling such as you suggest. The latest entry among the States is Virginia. Just this past 1989 session in Virginia, yet unsigned by the Governor, a statutory requirement was legislated that all persons convicted of felonies involving sexual assault will be DNA profiled and the records kept for law enforcement purposes.

Senator SIMON. And how many States are there now? Maybe we will get that in the next panel.

Professor STARRS. There are about four or five States. I can check off Colorado, California. King County in Seattle, WA, has its own local ordinance. There are a number that have such legislation in the hopper at the present time. I would say that there are about 15 where it is either presently enacted or is in the hopper at the present time.

Senator SIMON. Are there civil liberties problems here for our citizens?

Professor STARRS. Well, there are clearly civil liberties problems of a constitutional nature, which I think are resolvable and solvable, with respect to obtaining samples for the purposes of testing from a known suspect. The Supreme Court of the United States has spoken long and hard on the question of the fourth amendment search and seizure issue.

Quite obviously, if we are talking about a sexual assault, one would be hard pressed to think the Supreme Court would approve of taking a sample of semen from a suspect under any circumstances, whether it be court ordered or not.

However, blood is perfectly adequate for DNA testing and there are many instances where blood can, under court order, be acceptable. In addition, the English, in their response to the IRA terrorism in Northern Ireland, are utilizing buccal swabs from the interior of the mouth, which is less intrusive even than taking blood, and we can get DNA from that situs as well, and even hair is less intrusive than utilizing blood and extracting blood.

So, I think that we can overcome the problems of the fourth amendment by the approach we take with respect to the samples that we want.

Senator SIMON. Finally, if I may ask the two of you this, if suddenly the President of the United States were to say to each of you, what should we do, how do we move ahead, how do you respond? We are talking about a remarkable breakthrough in the area of enforcement of the law.

How do we accelerate this in practical terms? If the President of the United States says to you, Professor Starrs, what do we do, what do you answer him?

Professor STARRS. Well, I would think that the first level of attention should be in developing some kind of a national DNA data profiling system such as the FBI has with respect to fingerprinting and, of course, that is going to take substantial resources. And, of course, it has to take considerable cautionary controls as to the use of such profiling information. But that is where I would put my interest with respect to DNA if I were asked for priorities.

Senator SIMON. Dr. Peterson.

Dr. PETERSON. I agree, and I think that as I mentioned before, the impact of forensic science today has been limited by the limited resources, and DNA represents a tremendous breakthrough and we should certainly support and advance that technology.

But there are many other areas of forensic science in addition to DNA that need support. So, I think research, education, and training, not only of scientists, but of the legal user of this information, and support of programs to ensure quality assurance with respect to how this technique is applied—those would be the areas that I think merit foremost attention.

Senator SIMON. All right. We thank you both very, very much for your help here today.

Our next witnesses are Roger T.—and you are going to have to help me pronounce that name.

Mr. CASTONGUAY. Castonguay, Mr. Chairman.

Senator SIMON. Castonguay, all right, Assistant Director of the Laboratory Division of the FBI; and Mr. John Hicks, the Inspector-Deputy Assistant Director of the FBI.

Let me add here that on February 15, I sent a letter to Judge Sessions, the Director of the FBI, asking a number of questions about where we are. I have had my staff go take a look at the FBI headquarters and see what is happening. We are trying to find out where we ought to go in, and it really does look like the possibility of a very exciting development in this whole area.

Mr. Castonguay, we will let you start, and I hope I am not the first one to stumble over the pronunciation of your last name.

PANEL CONSISTING OF ROGER T. CASTONGUAY, ASSISTANT DIRECTOR IN CHARGE, LABORATORY DIVISION, FEDERAL BUREAU OF INVESTIGATION; AND JOHN W. HICKS, DEPUTY ASSISTANT DIRECTOR, FEDERAL BUREAU OF INVESTIGATION LABORATORY

STATEMENT OF ROGER T. CASTONGUAY

Mr. CASTONGUAY. One of many, Mr. Chairman. Thank you.

My statement will be an overview of the FBI laboratory and where DNA fits in——

Senator SIMON. If I may interrupt, we will enter—for all witnesses, we will enter the full statements in the record, and however you wish to summarize is up to you.

Mr. CASTONGUAY. Yes, sir.

The FBI laboratory is the only full-service Federal crime laboratory in the United States, and the Director of the FBI is charged by the Congress, and I quote from the Code of Federal Regulations, Mr. Chairman.

> To operate the Federal Bureau of Investigation Laboratory, to serve not only the Federal Bureau of Investigation, but also to provide, without cost, technical and scientific assistance, including expert testimony in Federal or local courts, for all duly-constituted law enforcement agencies which may desire to avail themselves of the service.

The services provided include scientific and technical support to investigations, examinations of physical evidence, court testimony, and forensic science research and training.

Direct scientific and technical support is provided to ongoing FBI and DEA investigations. These services have traditionally been performed only within the United States, but in recent years have been expanded to our extraterritorial jurisdiction provided by the Congress to include assistance with cases involving terrorist activities and other crimes against U.S. citizens and interests abroad. Examples of support to investigations outside the United States in the recent past include the bombings in Beirut, Lebanon, the hijacking of the cruise ship *Achile Lauro,* the murder of DEA agent Enrique Camarena in Mexico, and airplane hijackings and bombings in various parts of the world.

Support is frequently provided to crime scenes in major FBI cases and sometimes to State and local law enforcement in cases which achieve national recognition or involve expertise not available to the local jurisdiction. An example of this field support occurred during riots at the Federal penitentiaries at Atlanta and Oakdale last year. The support from our laboratory included assistance from the Special Photographic Unit, which provided surveillance photography to identify the riot ring leaders and facility survey photography to assist in contingency planning for hostage rescue. Forensic experts were also among the first agents into the prisons after the riots to identify, collect, and preserve evidence of crimes which may have occurred during the riots.

The research program concentrates on developing new methods for forensic examination and to build the scientific pedigrees required to support the introduction of new techniques into the courts. An aggressive research program was initiated to develop DNA technology for implementation by State and local crime laboratories, as well as by the FBI laboratory. The forensic analysis of DNA, the genetic material contained in living cells, offers the promise of a positive personal identification of an individual based upon the analysis of hair, a single drop of blood, or other body fluid, as explained by previous witnesses.

The laboratory's training program is designed to provide the best forensic science training possible to Federal, State, and local crime laboratory personnel and to law enforcement personnel. Many of

the specialized courses offered to State and local crime laboratory examiners are not available from any other source in the United States.

A variety of graphic and photographic services are provided, which include artist conception drawings, photographic field support in complex investigative situations, photographic processing, demonstrative evidence, and the production of special investigative devices.

The FBI laboratory also manages the polygraph program for the Bureau, the foreign language services program, the field photographic program, and the National Bomb Data Center. Through the Bomb Data Center, the laboratory manages the Hazardous Devices School at the Redstone Arsenal in Huntsville, AL. This is the only school for civilian law enforcement personnel in the United States which teaches render-safe techniques.

Our primary objective is to maintain a highly professional and thoroughly competent team of forensic experts with a broad range of capabilities to support the varied needs of the Nation's criminal justice system. The FBI laboratory is a national resource which has earned a reputation for excellence based on over 50 years of quality service.

Senator SIMON. Thank you very, very much.

Mr. Hicks.

STATEMENT OF JOHN W. HICKS

Mr. HICKS. Mr. Chairman, you have the statement that I have provided. It does provide a very comprehensive review of the steps the FBI has been going through in order to try to make this technology available to the law enforcement community. If I may, I will just summarize those things.

Senator SIMON. That would be appreciated.

Mr. HICKS. Yes, sir.

We have had essentially a four-phase program that has been underway since about 1985 as this technology came to our attention. The first phase was a research and evaluation phase in which we tried to identify the various sources of the technology, and then to establish contact with various research centers, gather information, and then try to take those methods which seemed to be most applicable to the types of problems that we encounter in a forensic laboratory and then to optimize those methods for our own use.

That process was carried out with a dedicated team of our own professional staff. At any one time, we had as many as 15 of our own professional staff scientists focused on DNA technology at our research center at Quantico.

Their efforts were supplemented through collaborative research programs where we had technicians from State and local crime laboratories come and work at our center with our scientists.

Through that process, we have satisfied ourselves that the tests can provide consistent, reliable results when applied to forensic-type specimens. We are comfortable that we can address the kind of questions that the courts would be concerned with in terms of whether or not these technologies should be accepted as evidence in court.

The research is continuing. The technology is changing very rapidly. We are literally at the cutting edge of the technology, and we anticipate that within the next few years we will see additional changes in DNA typing.

The second phase of our FBI program has been a phase where we have been trying to take these methods and apply them within our own crime laboratory in Washington. We did open our crime laboratory in October of this past year. We actually began examining evidence in criminal cases from throughout the country in December of this past year.

We have about 130 cases within our laboratory right now with requests for DNA typing. We have completed the probings, the typing, on about 45 of those cases. The majority of those are homicide or rape cases, violent type crimes. The majority also come from State and local jurisdictions. Only seven of those cases we have in our laboratory now are FBI cases.

Of the 45 cases we have completed the probings on, 6 of the cases actually provided information which eliminated the suspect as being the contributor of the blood or the semen in the particular case at hand.

I think it is important to point out, too, that those cases did go through a prescreening process using the conventional blood typing systems that have been in use in crime laboratories over the past several years, and through those systems they were not able to eliminate the suspect. So we think that DNA did provide an additional bit of very important information in those circumstances.

The third phase of our program has been a technology transfer phase where, through our research center at Quantico, we have conducted a number of activities. We have published a number of materials.

We publish a document called "The Crime Laboratory Digest," which is circulated primarily to crime laboratories, but it also goes to other scientists throughout the country. And different issues of this publication have been directed at different groups.

For example, one issue was targeted primarily to technical personnel where we tried to identify the types of methods that we are looking at, and also where we provided an overview of our research to date on those methods.

One of the other issues was focused at the crime laboratory managers in the various police departments throughout the country, and provided them with the kind of information to assist them in their decisions on what they will need in terms of resources—the equipment necessary, the space changes necessary, the availability of probes and other testing reagents. So through those publications, we have tried to make the community aware of where the technology is going.

I have already mentioned our visiting scientists program where we have personnel from State and local crime laboratories who have spent 4-month periods of time working in collaboration with our staff scientists.

Through that process, they have contributed to the validation work necessary, but they have also gained a proficiency of their own that they can carry back to their individual State laboratories.

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We have conducted our first DNA technical training course, which was a 4-week course conducted this past January. That was attended by about 40 technical personnel from State and local labs. They represented about 18 different States, about 20 departments.

So, through that training course, with some additional academic type of training, we believe that they can carry this technology into their local laboratories. We have found that the technology, the technique itself, does transfer very readily. These people were able to successfully perform the tests and get the proper kind of results.

We also have hosted international symposia where we will invite scientists from all over the world. We have invited such people as Dr. Alec Jeffreys, who is credited with introducing this technology to law enforcement.

Our second symposium is going to be held in June of this year. We expect about 300 people from throughout the world to attend and to share information as to where we are with the technology.

The last phase of the FBI's program is a phase that we are really just beginning to take a look at, and that is the standardization issue, primarily for the purposes of looking at the types of data banks that might be useful for law enforcement.

Several of these applications have been mentioned. One of the types of data banks we see as being necessary for the crime laboratory community is a population data bank, which has information in it that does not identify individuals, but simply identifies groups of individuals and gives the frequency of seeing certain variations in these DNA types within those populations. That information is necessary for interpretation purposes when we make our identifications with the DNA test results.

We also can see a very important application in trying to take information from the type of crime scenes where an individual is moving randomly from one jurisdiction to another, commits a crime, perhaps a sex crime, and then moves on—the Ted Bundy type of a crime. If we could classify evidence recovered at these crime scenes by the DNA types, we could capture this information in a computer, compare it between different jurisdictions, and help to identify related offenses for the benefit of investigators so that they could focus their investigations, coordinate their investigative effort and hopefully identify these perpetrators more quickly in the process to bring them to justice.

And as was also mentioned, there have been several States which have passed and proposed the type of legislation which allows them to collect blood standards from convicted offenders in certain categories.

Initially, it was in the category of sex offenders, but that has been expanded in many States to include offenders of other violent types of crimes, homicide or assault-type cases.

This file would be available as an investigative step in the event that individual is released on probation or parole, the investigators would have a file where they could check to determine if a prior convicted offender was again active in that type of crime.

In terms of standards, for any of these types of files to work, we have to have a standard classification, a nomenclature system for

DNA typing. There are many different types of procedures that can be carried out to get a DNA identification.

So, we have invited a group of representatives from crime laboratories throughout the country. We have also invited representatives from the Royal Canadian Mounted Police Laboratory to participate with us to try to agree upon the standard procedures necessary which would support this type of a data basing idea.

We expect that that work will be done near the end of this year, and once standards are established, we can explore the establishment of these other various types of data bases to support the law enforcement mission.

[The prepared statement of Mr. Hicks follows:]

STATEMENT OF JOHN W. HICKS
DEPUTY ASSISTANT DIRECTOR
FBI LABORATORY

FOR PRESENTATION BEFORE THE
SENATE COMMITTEE ON THE JUDICIARY
SUBCOMMITTEE ON THE CONSTITUTION
HONORABLE PAUL SIMON, CHAIRMAN
MARCH 15, 1989

SUMMARY

FBI scientists working at the Forensic Science Research and Training Center located at the FBI Academy, Quantico, Virginia in collaboration with scientists throughout the world have evaluated many different DNA probes and procedures and have completed studies designed to establish the reliability and validity of DNA technology.

The FBI Laboratory has established a DNA Analysis Unit to process forensic evidence submitted in criminal investigations and began casework in December, 1988. Approximately 120 cases have been accepted for processing from Federal, State and local law enforcement agencies.

An ambitious program is underway to transfer this technology to state and local crime laboratories through collaborative research projects, technical training courses, other seminars and publications.

Just as the swirls and patterns of fingerprints are unique markers of specific individuals, DNA typing provides unique patterns that can also be useful for individual identification. When properly used, DNA typing has the potential for identifying the violent criminal offender sooner than previously possible and for clearing innocent persons who may have been regarded as suspects in a particular crime. FBI Director William S. Sessions has charged the Bureau with providing the leadership necessary to ensure DNA technology is effectively and responsibly applied to assist all levels of law enforcement, thereby enhancing the nation's effort to combat violent crime.

Deoxyribonucleic acid (DNA) is an organic substance found primarily in the nucleus of living cells. It comprises the chromosomes within the nucleus and provides the genetic code which determines a person's individual characteristics. The code is expressed by the arrangement of four basic building blocks, called nucleotides which are represented by the letters A (adenine), G (guanine), C (cytosine), and T (thymine). These nucleotides are linked in chain-like sequences and their order can vary to provide an almost infinite number of possible arrangements. There are about 3 billion nucleotide bases in the entire human genetic code.

The FBI Laboratory began its first scientific inquiries into DNA technology in late 1984 with a collaborative project with the National Institutes of Health. Developments in the private sector caused us to accelerate our efforts. Beginning in December, 1986, our scientists made numerous site visits to

private DNA testing laboratories in the United States and England, as well as to other institutions engaged in DNA research. This resulted in a decision in July, 1987, to organize a dedicated research team at our Research Center at Quantico, Virginia, to develop this technology for use in the crime laboratory. An ambitious research program was developed and we believe it has been highly successful to this point. The research focus has been on (1) optimizing extraction procedures tc improve the recovery of analytical quantities of DNA from forensic specimens; (2) streamlining test procedures to reduce analysis time; (3) improving the sensitivity for detection of small amounts of DNA; (4) keeping down the costs of reagents and supplies required for DNA testing; and (5) expressing the results of the DNA test in a form which is easily interpreted by the analyst. Our researchers, in collaboration with scientists from other laboratories in this country and abroad, have evaluated many different DNA probes and procedures and have conducted studies on samples taken from actual evidence items.

In April, 1988, we began training FBI personnel from our operational Laboratory in Washington, D. C. These personnel were experienced forensic specialists proficient in the traditional blood grouping and protein testing methods now in wide use by crime laboratories. Their training included enrolling in a short course in molecular biology arranged through the University of Virginia, a series of lectures presented by members of our research staff and a period of instruction and practice with the

technique itself. With this training, these technicians have demonstrated they can successfully perform the DNA analysis and can understand and clearly articulate the processes at work.

While our personnel were being trained, we designed a DNA testing laboratory and made necessary renovations to existing laboratory space. The trained DNA personnel assumed occupancy of the new DNA Laboratory in October, 1988. A validation program was carried out in order to assure that the DNA analysis methods and examination protocols performed properly in the new Laboratory. This "shake-down" process included a series of prepared proficiency samples which simulated casework materials being processed by each technician in the DNA Laboratory. The final validation process was completed in December, 1988, and the FBI Laboratory began DNA extractions from selected casework materials at that time. We are aware that similar programs for DNA implementation are underway in crime laboratories in Maryland, Virginia, North Carolina, and New York and that many other states are preparing for such programs.

The DNA test method to be employed initially will be what is known as the RFLP procedure using a series of selected single locus probes (also called variable number tandem repeats or VNTR). Work is continuing at the FBI Research Center to develop and/or evaluate other DNA typing procedures for law enforcement use. These projects include the development of non-isotopic markers for DNA probes, further work with a DNA amplification process called polymerase chain reaction (PCR), the utility of

mitochondrial DNA (as compared with chromosomal DNA), and an evaluation of direct sequencing processes for use on forensic specimens. It should be pointed out that the only information which can be obtained by the DNA test methods in use or under investigation in the FBI Laboratory are the unique patterns which can distinguish one individual from another. The tests do not recognize any known functional inherited trait or characteristic and cannot be used to diagnose disease conditions. The tests specifically respond to what are referred to as non-coding regions on the DNA molecule and the results are expressed with reference only to allele frequencies in a given population; that is, how frequently a particular DNA pattern developed with a particular DNA probe is likely to appear among different individuals.

I have described above the program we undertook to develop DNA technology and implement its use in the FBI Laboratory. At the same time, we are committed to supporting state and local laboratories in their efforts to implement DNA testing on evidence specimens. In June, 1988, the FBI hosted a seminar on DNA technology at the Forensic Science Research and Training Center. The program included presentations by key scientists from academia, private sector laboratories, other DNA research centers and the international crime laboratory community. Various aspects of the technology were addressed and there were also discussions on computer data systems for DNA profile information and the use of standardized testing protocols. Standards and data banks will be commented upon later in this statement.

The "Crime Laboratory Digest" is an FBI publication printed quarterly and is used to disseminate information of interest to the crime laboratory community. Several issues of this publication have focused on DNA technology. The January, 1988, issue described for laboratory technicians the methods of DNA analysis under investigation in the FBI Laboratory. A supplemental issue was circulated in April, 1988, which provided crime laboratory managers with information on the equipment necessary to establish a DNA testing capability and considerations for setting up the laboratory facility and training of personnel. The October, 1988, issue provides a summary of the FBI's research and DNA validation studies. These publications have been circulated widely in the forensic community and copies are made available upon request directed to the FBI Laboratory, Washington, D. C. 20535.

There are two other important FBI programs to facilitate the transfer of this technology to state and local laboratories. One is the visiting scientist program which enables scientists from state and local crime laboratories to work at the Forensic Science Research and Training Center in collaboration with FBI research scientists. This four-month program began in July, 1988, and is designed to provide the technical personnel to address the validity and reliability issues associated with DNA testing as quickly as possible. On completion of the program, the participating scientists have developed a high level of technical proficiency in DNA test methods and valuable experience to aid

their efforts to apply the methods in their own jurisdictions. There are currently 16 crime laboratory systems scheduled for participation. The second program consists of a four-week specialized technical training course on DNA methods which was conducted in January, 1989, and will be offered again in June, 1989. This course, in conjunction with other studies, will also enable technicians to develop DNA testing programs at the local level.

The use of DNA technology in law enforcement offers tremendous potential in providing investigators with a powerful tool to help solve their most difficult cases involving violent crime. Using DNA, the crime laboratory may be able to determine that the same rapist is responsible for a series of assaults in several different jurisdictions. As suspects are developed by investigators, they may be exonerated through DNA tests and investigators may redirect and narrow their search for the perpetrator. Several states are considering collecting DNA identification profiles on certain categories of convicted offenders. Just as latent fingerprints from a crime scene may now be searched through fingerprint records maintained by police agencies, the DNA profile from evidence left at a crime scene could be searched against the DNA profiles of convicted offenders who may have been released on probation or parole. In another potential application, the DNA profile from the remains of an unidentified deceased might be retained for comparison with the DNA profiles of the parents or other blood relatives of missing

persons in order to resolve the identification. These kinds of files could be efficiently handled through computer automation; however, it should be emphasized that the computer data bank would be nothing more than an information management and screening tool. When, based on a computer search, it is determined that an identification is indicated between individual DNA profiles, the evidence specimens, as well as the specimens from the suspect, should, ideally, be reanalyzed simultaneously and a direct confirmation made.

For the full law enforcement potential of DNA profiling to be realized as described above, there must be a cooperative effort nationwide to build data files with the appropriate DNA information. There must be uniformity within the crime laboratory community on the DNA test methods used so that the profiles developed can be effectively catalogued and compared and there must be established the quality controls necessary to protect the integrity of the information in such files as well as controls on security and access. There have already been some discussions within the forensic community exploring the feasibility of DNA data banks and uniform test protocols. A technical review group has been assembled which is comprised of representatives from several state and local crime laboratories and includes advisors from academia and other research centers. This group began its discussions at the FBI's Research Center in November, 1988, and is in the process of evaluating various protocols and offering recommendations for uniform protocols necessary for the

establishment of computer data files. The observations and recommendations of this technical review group will be presented to the forensic laboratory community in appropriate forums.

In summary, we see the forensic use of DNA technology as an extremely important new scientific tool for law enforcement and have made a strong commitment to facilitate its use by crime laboratories nationwide through programs at the Forensic Science Research and Training Center. Through research conducted at the Center and through collaborations with other scientists and forensic specialists in the United States, Canada and England, we are satisfied that DNA testing using RFLP methods can be successfully performed by competent and properly trained technicians, can provide reliable results which meet the needs of the criminal justice system and which satisfy the requirements of the courts. The use of DNA technology in law enforcement when applied in a responsible and well-coordinated manner with due consideration for the needs of our criminal justice system will enhance the effectiveness, efficiency and productivity of police agencies at all levels.

Senator SIMON. You will have to decide among each other to whom these questions should be referred, but you mentioned establishing standards. You mentioned bringing in Canada. The British have been in the lead in this.

The standards that you are talking about, will those be international standards that people around the world could adhere to and that we could exchange information about?

Mr. HICKS. Well, at this stage of development, it looks as if we are going that way in terms of the North American continent. The British and some European laboratories have recently announced that they have established as a standard a particular restriction enzyme which is not, as it turns out, the restriction enzyme that we are focusing our efforts on.

We are in discussion with the British and with others in the European crime laboratory systems. Again, through discussion, we hope that eventually we will reach a worldwide standard, but that may not be possible.

Senator SIMON. In terms of numbers, what kind of numbers do we have in terms of a DNA identification now, or do you not have a file developed? Do you just provide information on a case-by-case basis, or where are we right now?

Mr. HICKS. Well, I am not certain I understand your question.

Senator SIMON. In other words, you have x number of million people on file in terms of fingerprints—230 million, or whatever the number is. Are we at the point where when you get requests, do we keep that information on file?

Mr. HICKS. That is correct; yes, sir. When we receive samples for analysis at this point in time, they are simply on an individual case basis where perhaps a blood sample from a suspect and a victim is submitted along with evidence materials.

We perform that analysis, and the only record that is maintained is the analytical record which supports the technician's work. We do not keep that separately filed, then, as far as having those DNA files on record by name of the suspect or victim at this point in time.

Senator SIMON. What kind of costs are we talking about on this significant breakthrough in law enforcement? To really get launched properly, what kind of money are we talking about needing here?

Mr. HICKS. Well, within our laboratory, our experience to this point is it costs us about $30 a sample to perform the analysis for supplies. We have to also include in that—there is an investment cost up front that is needed for the equipment and the space to do the analysis which is fairly nominal. It is not an extraordinary cost in terms of the other types of expenses for other equipment in a crime laboratory.

So, we believe that within our laboratory, at least, the existing resources are adequate to enable us to provide this service.

Senator SIMON. But you are talking before long about creating, in fact, a file, and if you are talking about 30 apiece, if you are going to have a file just of Federal prisoners, you are talking about a fairly substantial amount of money as you move beyond that.

Now, obviously, State governments will be collecting a lot of the data and presumably sending those files to you. So before very

long, we are talking about some kind of increment in funding to take care of this, are we not?

Mr. HICKS. Well, again, it is very early in the process here to determine that, but the way we envision these types of convicted offender files, we would expect that the work will be done at the State level.

The jurisdictions that are passing legislation—we would expect that they would perform those tests on those convicted offenders and that they would retain that information. The role that we see at this point in time for the FBI would be, through perhaps a national telecommunications network, to support the exchange of that information between various jurisdictions.

That network is in place right now with the National Law Enforcement Telecommunications System and the NCIC system. That framework may be useful in facilitating the exchange of this information between different jurisdictions.

So, for a centralized file for the FBI, we see very limited numbers of applications. Perhaps the serial offender type file, the random killer type file—it would seem useful to put that in a centralized location. But, again, it is very early and I really don't have a good answer for you.

Senator SIMON. But you see ultimately, then, each State collecting that individual file, and if a murder occurs and you have a blood sample, that simply would go to the 50 States and say this is a murder that occurred and you have this DNA profile in your files.

Mr. HICKS. Yes, sir; for example, if a State—let us say that California has established such a file of convicted offenders, and perhaps in Oregon, a neighboring State, a crime is committed. They may then query the California system to ask whether or not the DNA type that they established in that crime may have been the same type as of a prior offender.

Senator SIMON. One final question and then I want to yield to Senator Specter here. Let us say I get a blood transfusion. Do I have two DNA profiles in my system or does the new blood take on the composition of the old DNA profile? Where do I stand on that?

Mr. HICKS. Well, at least initially for the analyst, it will produce a confused result, and the analyst will recognize that there are more bands here than he should be seeing in the particular analysis. But the body, through its own metabolism, will adjust and will return to its normal state over a period of time. I guess it depends on the individual metabolism as far as how long that is. It may be a matter of weeks or perhaps a few months.

Mr. CASTONGUAY. With the existing—I am sorry, Senator.

Senator SIMON. Yes, Mr. Castonguay.

Mr. CASTONGUAY. With the existing processes, it would be a similar process to what they go through now when we have mixed body fluids. So you have a crime scene with evidence that comes in with torn shirts, bloody shirts, panties. It has a mixture of body fluids and they have to separate out the various DNA and they would come up with a mixed result. They would have the same process to go through with the transfused blood.

Senator SIMON. And you could tell that I had a mixture by testing me?

Mr. CASTONGUAY. Yes, sir.

Senator SIMON. All right.

Senator Specter.

Senator SPECTER. Thank you, Mr. Chairman. I regret that I have not been able to be here earlier and will have to leave shortly. We have Secretary of State Baker before a foreign operations subcommittee. For those who don't know, you may wonder why more Senators are not present or why Senators come in for a very short period of time, but the scheduling of committee hearings is just extraordinarily complicated.

I did want to stop by for just a moment to express my view that this is a remarkable and extraordinary device for ascertaining identities which I will study with care. I will review the notes today and have staff present.

I know from my experience as a district attorney, identification is frequently the toughest item. You may have hair samples and they may be similar or consistent, but they do not rule out other people. Or you may have blood samples and it is consistent with that of the defendant, but it is also consistent with the blood sample of many, many other people.

If we are really able to have a device for ascertaining with precision who the person is, it is really revolutionary both in terms of convicting the guilty and assuring the juries that you have the right person, and also in acquitting the innocent. Nothing is more difficult in the course of a criminal trial than the question of identification.

The case books are full of situations where people have been inaccurately identified, and the standards as to what constitutes enough evidence to go to a jury on the question of identification are very involved.

I recall once as an assistant DA trying a case where a defendant wore a stocking over his head; we knew it was a man. In a struggle, the stocking was pulled up right to the level of the nose, and I thought the identification was insufficient as the prosecuting attorney.

The trial judge let the case go to the jury and I asked for an acquittal in the case because I thought there was insufficient evidence of identification. It later turned out that we, in fact, did have the wrong person.

So, if we are able to find a method for positive identification, it is a really remarkable discovery and one which we should pursue. I thank you very much for offering your testimony and, again, I express my regrets that I cannot stay longer.

Senator SIMON. I thank you for being here, and you are correct. I am in the unusual situation of having four subcommittees meeting right now, and I don't know what we do about it.

Senator SPECTER. Well, one thing that can be done about it is that if the majority and minority status of the parties are changed, you won't be the chairman and you won't have to stay. [Laughter.]

Senator SIMON. I don't favor that solution, Senator Specter. [Laughter.]

We thank both of you very, very much. We would like to keep in touch with you, too, because, obviously, we are just in the initial stages of something—Senator Specter calls revolutionary; I also

think it is revolutionary. It is probably the most significant development in terms of law enforcement since the fingerprint was identified as a law enforcement tool. I thank both of you for your help here and we intend to be back in touch with you.

The final witness is Jeffrey L. Ashton, the assistant State attorney of the Office of the State Attorney from Florida.

Mr. Ashton, we are happy to have you here.

STATEMENT OF JEFFREY L. ASHTON, ASSISTANT STATE ATTORNEY, STATE OF FLORIDA

Mr. ASHTON. Thank you, Mr. Chairman. My first knowledge of the technique which has come to be known as DNA identification testing——

Senator SIMON. Do you want to pull the microphone just a little closer to you there?

Mr. ASHTON [continuing]. Was in 1986. It involved news stories——

Senator SIMON. I think we may be having trouble. If you don't mind taking that other chair, I think the mike is working a little better over there.

Mr. ASHTON. How is that?

Senator SIMON. Good.

Mr. ASHTON. My first knowledge of the technique that has been discussed here came in late 1986 when I heard and read news reports, as I am sure many other people did, about a case in the United Kingdom involving the rape and murder of two young girls that happened approximately 2 years apart.

One of the investigative techniques that was used was used with the help of Dr. Alec Jeffreys that you have heard mentioned here today. The technique was to take blood samples from approximately 4,000 individuals in a particular town called Enderby in England.

The purpose was to try and determine whether one of those individuals was, in fact, the person that had raped and murdered these two girls. The technique ended in finding a suspect not in the way that was intended. Apparently, the suspect convinced or bribed another individual to go in his place and give a blood sample, and therefore he was identified in that regard.

I noted this with a great deal of interest, having prosecuted many rape cases in my career, and I hoped that one day that technology might be available here in the United States.

In approximately July 1987, I happened to be looking through a lawyer's publication and saw an advertisement for a company called Lifecodes that advertised a paternity testing service involving genetic identification. I thought perhaps the two might be the same. I contacted the laboratory and, in fact, was informed that they did do identification testing for forensic purposes.

At that point, I did not have a case that I felt it would be helpful for, but I knew that whatever case we were going to do this in needed to be something significant. It needed to be a case where identification was the only issue and a case where we could convince the court and our own office to spend the money to break into this new area.

I had prior to that been in contact with another prosecutor in my office by the name of Tim Berry who was prosecuting a man named Tommy Andrews. Mr. Andrews was charged with six separate, very violent rapes, all of which were very similar.

Women would be home at night alone. An individual would break into their house in the middle of the night, would attack them. It was a black man. Usually, he would attack them in the dark and cover their heads so that they were not able to identify him.

I knew that in one case, we had somewhat of an identification, a brief glimpse, and in one case we had a fingerprint on the outside of a house. So I knew that it was important.

I went to Mr. Berry and we decided to give this a try. At that point, we could find no other evidence that anyone had ever done this before, but we felt it was worth a try. With the help of Lawson Lamar, who was then the sheriff and now a State attorney, we were able to send the samples and have them analyzed.

The analysis came back positive on two of the six samples. The report was that Tommy Lee Andrews was, in fact, the person that was the source of the semen found during the rape exams of two of the victims. We then set the cases for trial.

In November 1987, we tried Mr. Andrews for rape, burglary, and related offenses. We presented the evidence of DNA identification testing. We used a laboratory in New York called Lifecodes, which is the other laboratory in addition to Cellmark. They sent experts down, two experts.

We also asked for the assistance of Dr. David Housman, who is an expert geneticist fairly well known nationally, to help us present the evidence to the jury. With their help, we were able to present the evidence to the jury, got the judge to allow it in under the applicable standards, and argued to the jury.

The particular case that we had, the DNA was really the crucial piece of evidence. The only other evidence we had, or identification, was a fingerprint on the outside of a window screen and we were unable to really establish when it got there.

The jury, after 2½ hours of deliberation, returned a verdict of guilty on the rape charges and the other charges. And so far as we have been able to tell, this was the first recorded conviction in the United States or, in fact, anywhere in the world, as far as we know, using DNA identification.

I wanted to comment that your chart is about a year off. It was November 1987 and not 1986. Anyway, that basically was our first involvement with it. Since then, in October 1988, the Fifth District Court of Appeals in Florida has reviewed the action of the trial judge in allowing the evidence, has ruled in a rather lengthy opinion that the evidence is admissible, that it does supply the necessary predicate for any standard of admission.

At this point, we are pursuing work on DNA identification not only in rape cases where it is most obvious, but in homicide cases. As I think Dr. Peterson mentioned, homicide cases—it can be helpful in identifying blood samples; say, a victim's blood on a defendant's clothing or a defendant's blood at the scene of a crime.

It is also helpful in identifying bodies. Many times, samples can be taken from bone marrow or other bodily substances to identify a body that is not otherwise identifiable.

We are also using DNA in one other case where we have a homicide without a body. We have a missing person; we have a car that is his. We have a great deal of blood, clearly indicating that whoever shed that blood must be dead, but we have no body. And we are exploring the possibility of identifying the source of that blood as being the victim, and therefore identifying him as being dead.

Thank you.

Senator SIMON. I thank you. When you contacted this laboratory for the information, what did that laboratory charge you?

Mr. ASHTON. I believe in the *Andrews* case, they charged us—for the two cases where we got positive results, they charged us $600 each. Basically, we sent them the rape sample kit which contained a swab of semen from the victim, a sample of the victim's blood, and a sample of Mr. Andrews' blood. And to do that entire analysis, they charged us $600 for each case.

For the cases where no result was obtained, I believe they charged us $75 apiece for those.

Senator SIMON. Will the State of Florida eventually have a lab of its own to analyze DNA evidence? Where do we go as a nation in this whole field?

Mr. ASHTON. Well, the State of Florida, through the Florida Department of Law Enforcement that runs our crime labs, is actively working on getting a crime lab going in Florida—not a crime lab, but a DNA lab going in Florida. The last estimate I had was that it may be up and running some time either later this year or early next year. So we are hoping to get that done.

I just learned at the committee hearing today that the FBI has their laboratory up and running, and that is certainly something we are going to be very pleased to hear, to have another laboratory that we can submit these samples to.

Senator SIMON. If you don't have this information, maybe I can ask Mr. Hicks or someone else. Are there any States that now have laboratories operating?

Mr. ASHTON. There is one that I know of. I know the State of Virginia does according to—I spoke to their lab director some time ago and he told me that they did. I don't know of any others.

Senator SIMON. Yes, Mr. Hicks.

Mr. HICKS. The State of Virginia has indicated they will open their operating facility in May of this year, so they are very far along. Nassau County in New York State is also very close to coming on line with this technology. But those are the only two that I am aware of at this point.

Senator SIMON. You heard my questions to the two officials from the FBI. Does the procedure of having the State laboratories and then coordinating information between States make sense, or should we have a central FBI file on this? How should we proceed?

Mr. ASHTON. Well, I would hope to see the FBI as a central repository of information, not necessarily that all the tests be performed by the FBI, because I think every State is going to have its own laboratory to fully utilize the technology.

Once the laboratory is in place, those tests can be done by that laboratory, but the FBI would be a very important central repository of information. I think Mr. Hicks mentioned Ted Bundy, and Ted Bundy obviously was recently executed in Florida, so he is on our minds quite a bit.

This technology can be used not just for nationwide serial killers, but my experience is that most rapists of the type that we deal with like Mr. Andrews do rape a number of times even in one community. And I think DNA is valuable even where you don't have a suspect merely to show that, in fact, you have one person doing all these different crimes, and therefore investigation can focus in more clearly.

Senator SIMON. And you heard me ask two of our witnesses earlier how they would respond if President Bush asked them what to do. What would your response to that question be?

Mr. ASHTON. Really, two things, I think, which echo what you have already heard. One is in some way to promote research. The only real problem we have now with the DNA science, and it is really in its infancy to some extent, is we need to be able to get results from smaller and smaller samples.

The biggest frustration that we have in law enforcement is that we have a sample, but we get a report back from the laboratory that there is not enough there. And I think with development of technology, we will get to the point where we can get a result with smaller samples.

Senator SIMON. May I interrupt this first point? When you say there is not enough there, my understanding is if you get one drop of blood, you have enough. Is that not correct?

Mr. ASHTON. No, sir; there is no set amount. You don't have to have 1 cc or 2 cc's, but we have had a number of cases where we have sent in samples and the result has been there isn't enough DNA in the sample.

The problem is there has to be more than one single cell. There must be, as I understand it, a number of cells, either white blood cells or sperm cells, in order to get a sufficiently strong reading to give you a result.

Senator SIMON. Are others of you in agreement with that? Mr. Hicks?

Mr. HICKS. That is essentially correct. One of the problems with DNA is that it is subject to bacterial degradation and environmental factors, so that it is possible over a period of time that the DNA fragments break down into such small pieces that you don't get any single bands.

Senator SIMON. And presumably as our technology improves, we probably can do more and more with smaller and smaller units?

Mr. HICKS. That is certainly true, sir.

Professor STARRS. I think it is also important to point out that DNA is hardier and more stable even with respect to degradation than blood and genetic marking systems that we now have. So it is even better, but we can improve upon what we now have.

Mr. ASHTON. That is true. There have been, I believe, reported cases of DNA being found in mummies. I know I read a report of that. So it is something that has the potential of being much hardier than the standard blood kind of serology that we do now.

Senator SIMON. And you were about to make a second point when I interrupted you here.

Mr. ASHTON. The only second point is really just to echo what has already been said. There needs to be standardization because right now we have two commercial laboratories that do this test. They do the test in different ways, so their results are not comparable.

In other words, if a test on an unknown sample is done at one lab and a test on a defendant's blood is done at another, you can't compare them. So, I think that standardization of a particular type of test is really helpful. And, again, that promotes the idea of a national bank where people in one State can refer to people in another, because we have found that crime really crosses State lines; it does not respect State boundaries.

Senator SIMON. I appreciate your testimony, and you are to be commended. If your boss were here, I would suggest you ought to get a raise, but you can convey that to him.

Mr. ASHTON. I will tell him.

Senator SIMON. All right. [Laughter.]

Mr. ASHTON. Thank you.

[The prepared statement of Mr. Ashton follows:]

OPENING STATEMENT OF JEFFREY L. ASHTON
BEFORE THE SUBCOMMITTEE ON THE CONSITITUTION
MARCH 15, 1989

My first knowledge of the technique which has come to be known as DNA identification testing came late in 1986. Along with many others I read with interest reports of the use of this new technique in the United Kingdom during the investigation of two brutal murders of young women in the town of Enderby. The test was performed on blood samples collected from approximately 4,000 men who lived in this small community. The purpose being to locate the man who had raped and murdered these two young women. I noted this new development with interest and a hope that one day such technology would be available here in the United States.

In July of 1987 an advertisement in a state lawyer's publication in the State of Florida caught my eye which advertised paternity testing using a new genetic technique. The advertisement was titled "He's Wearing His Daddy's Genes" and pictured a drawing of an infant. The possibility occurred to me that this testing that was advertised might be the same as that I heard of from England so I contacted the company to inquire as to the availability of forensic testing. I was told that such tests were available and were performed by a company called Lifecodes that was located in New York State. I felt that the case which would test the admissibility of such new evidence would have to be a very special case. At that time Tommy Lee Andrews was awaiting trial on six separate charges of rape and related offenses. Though I was not the prosecutor assigned to the cases I knew that they were serious cases which involved questions of identity. I contacted the prosecutor assigned to the cases, Tim Berry, and we decided to determine whether such a process would reveal helpful evidence in Mr. Andrews' cases. With the help of Lawson Lamar, then Sheriff of Orange County, presently the State Attorney in Orange County, we were able to quickly transport the samples to the labortory in New York. The samples which were sent consisted of swabs taken from the victim at the time of the rape examination, a blood sample from the victim, and a blood sample from the suspect, Tommy Lee Andrews. Six separate cases were sent to laboratory in New York and to our great pleasure, two of the cases returned with positive results. In both cases the results positively identified Tommy Lee Andrews as the donor or the semen. At that point Mr. Berry and I set about the task of preparing Mr. Andrews' cases to be the test case in the State of Florida in the use of DNA identification testing. At that point we were unaware of any other prosecutors in the United States who were working along similar lines.

In November of 1987, trial was held in the Circuit Court in Orange County, Florida, in the case of State of Florida vs. Tommy Lee Andrews. This case involved a break-in and rape of a young woman in Orange County, Florida. The woman was attacked as she slept in her bed in the middle of the night, in the dark, by an unknown assailant. During the rape her face was covered and, therefore, identification of the assailant was impossible. Crimes scene investigation in that case revealed that a window screen had been removed from one window in the house which appeared to be the point of entry. On that window screen were

found two fingerprints which were later identified as being those of Tommy Lee Andrews. Though the fingerprints on the screen were clearly those of Andrews there was no reliable method of determinging when those fingerprints may have been left of the screen. The evidence of DNA identity testing was given to the jury by an expert imployed by Lifecodes Corp., the company that did the test. They also heard testimony from the technician who performed the test as to the exact procedure used and the quality of the samples that were used in that examination. They further heard from Dr. David Housman, member of the faculty of the Massachusetts Institute of Technology and nationally known genetic researcher, who informed the jury as to basic scientific principles behind the accuracy of DNA identity testing. Having heard two days of testimony on the case the jury retired on Friday, November 6th to consider their verdict. After two and one-half hours of deliberation the jury returned a verdict finding Tommy Lee Andrews guilty of Burglary, Sexual Battery, and Aggravated Battery. From all available counts, this is the first conviction ever obtained anywhere in the world using the results of DNA identity testing. Mr. Andrews was sentenced to 22 years in the Florida Department of Corrections for those offenses.

In February of 1988, Mr. Andrews was tried again for the rape of another young woman, again using the results of DNA identity testing. He was once again convicted and sentenced to 78 years Department of Corrections consecutive to the sentence in the previous case.

An appeal was taken Mr. Andrews' conviction in November 1987. That appeal was heard by the Fifth District Court of Appeals of the State of Florida which rendered an opinion in October of 1988. That opinion affirmed the conviction of Tommy Lee Andrews and approved the use of DNA identification testing in the criminal courts in the State of Florida.

Since that time the use of DNA identification testing has spread throughout the United States. At least eight States have to this point approved the use off DNA identity testing in criminal trials. To this point, however, none of those cases have found their way through the appeallate courts except for Tommy Lee Andrews.

DNA identity testing is one of the most valuable tools to a prosecutor and to law enforcement. It combines the attributes of both fingerprint evidence and standard seriological evidence. Like a fingerprint it allows for a positive individual identification of the assailant. Its advantage over a fingerprint is that a fingerprint cannot be successfully dated. In other words, a fingerprint can be left at a crime scene at any time. Also, a fingerprint is often not directly related to the crime. DNA identity testing draws its conclusions from samples of human cells. Typically, those samples of human cells are more closely related to the actual commission of the crime than a mere fingerprint. As an example, semen left as a result of a rape is a direct product of the crime as is a blood stain left at the scene of a murder. Also, in some instances blood and semen can be successfully dated, that is that an expert can determine within certain parameters how old the sample is. In rape cases in particular physicians by examining the semen itself and the condition of the source of the semen, that is the woman's vagina, can make general determinations as to how long the sample had remained since it was deposited. Similarly,

blood stains are something that is noticed and seen and can usually be dated by establishing how long they've been on the surface. The ability to date the sample has always been the maing value of standard seriology. But standard seriology has never been able to make an absolute individual identification.

DNA identity testing has potential uses in the prosecution of rape caes particularly cases of serial rape where numerous incidences involving different victims occurr and the police can use this type of testing to determine positively that one individual is responsible for all of the rapes. This evidence will allow law enforcement to focus its investigation more clearly when we have numerous assaults involving one defendant. This process also has potential uses in homicide investigations where we have blood of the victim in some relatative place, i.e., on a murder weapon, on the defendant's clothes, or on the defendant's car. In like fashion where we find the defendant's blood somewhere at the scene of a crime then that can clearly identify him as being present at the time the crime was committed. DNA identity testing also has value in both identifying bodies that are found in states of advanced decomposition by comparing samples of DNA taken from the body to either samples of that same person taken in life or to samples taken from relatives. DNA identity testing has also been used in situations where no body is, in fact, present. Such a situation might occurr where a great deal of blood is present clearly indicating a loss of life but no body is found. A sample of the blood can be taken and compared to the parents of the alleged victim to determine whether that blood came from one of their children. This method may allow prosecution in cases which could not be prosecuted in the past for lack of a body.

Senator SIMON. Let me just say this to our friends from the FBI: I would appreciate getting a quarterly update from you on what is happening in this area. And to other witnesses and to anyone else who is here and interested in this field, we are on the cutting edge of something that could be very, very important in law enforcement, and I would welcome any suggestions you have as to what we should be doing.

Our hearing stands adjourned.

[Whereupon, at 11:17 a.m., the subcommittee was adjourned.]

APPENDIX

ADDITIONAL SUBMISSIONS FOR THE RECORD

CELLMARK
DIAGNOSTICS

PATERNITY
Fee Schedule

DNA FINGERPRINTING℠ testing (Fresh blood sample)	$200/sample
Non-blood sample	$350/sample
Drawing	Charged by drawing station
Transportation	No charge
Telephone consultations	No charge
Expert witness fee	$500/day + expenses
Depositions	First 1/2 hour free of charge, then $150/hour + expenses for a Ph.D. $75/hour + expenses for a non-Ph.D.

TERMS OF PAYMENT

- All fees are payable in advance of testing.

PRICES SUBJECT TO CHANGE

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CELLMARK
DIAGNOSTICS

FORENSIC
Fee Schedule

Complete DNA testing	$350/sample
DNA extraction only	$150/sample
Processing of already extracted DNA sample	$250/sample
Processing of non-prepared sample (Actual processing time only)	$85/applied hour
Membrane probing (Membrane prepared by another laboratory)	contact Cellmark Diagnostics
Interpretation of autoradiograms produced by another laboratory	contact Cellmark Diagnostics
Telephone consultations	No charge
Expert witness fees	$1,000/day + expenses for a Ph.D. $750/day + expenses for a non-Ph.D.
Depositions	$150/hour + expenses for a Ph.D. $75/hour + expenses for a non-Ph.D.

Terms Of Payment

- An invoice for the fees due will be forwarded upon completion of the case.
- Terms are net 30 days.

Note: The above fee schedule effective November 15, 1988. PRICES SUBJECT TO CHANGE

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CELLMARK
DIAGNOSTICS

FORENSIC Testing Facts

The Procedure For Sending In Samples

Due to the uniqueness of each forensic case, a consultation with the Laboratory Director and/or laboratory staff is required prior to the submission of evidence. When the case is accepted for testing, the samples should be shipped via one of the overnight carriers or hand carried so that chain of custody can be traced. A telephone call informing Cellmark when the package should arrive will help to ensure its secure transportation. Knowing the date of the arrival will allow us to notify you immediately if there is a delay. Containers with samples should be sealed and clearly labelled with the date of collection and identification name and/or number.

PLEASE NOTE: Cellmark Diagnostics does not accept samples coming directly from the crime scene. In all forensic cases, the evidence collected *must* first be sent for examination to a forensic laboratory to determine the blood species (human or animal); with semen samples, to ascertain the presence of sperm cells; with hair samples, to verify whether or not the hairs have roots; and to perform blood typing and other standard tests. The results of this analysis should then be sent to Cellmark along with the evidence.

The evidence *must* be accompanied by a cover letter containing the following information:

1. what DNA testing is required and which samples are to be compared
2. list of samples and their identification numbers
3. condition of samples
4. race of the suspect(s)
5. a copy of the laboratory report
6. names and telephone numbers of contact persons
7. names and addresses of the persons who are to receive copies of the final report and the invoice
8. court date (if known).

Upon completion of the test all evidence is returned to the client by registered mail unless otherwise specified. For example, when we extract DNA from a stain, the cutout stain is returned. The stain no longer has DNA or enzyme/protein markers. The membrane, which contains the DNA sample, is stripped of the Jeffreys probes and is also returned. These membranes can be stored in plastic wrap (to keep the sample moist) in a refrigerator for months to years.

Reporting Of The Test Results

The processing time normally takes 4–8 weeks, starting when the samples reach our laboratory and depending on the specific nature of the evidence in the case.

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Sample Requirements

Whenever cellular material is left at the crime scene it could potentially serve as a source of DNA. The most frequent forms of forensic evidence available for testing are blood, semen, hair roots and post mortem samples. Since DNA is present only in nucleated cells in the human body, it will not be found in saliva except when epithelial cells are present or semen lacking sperm cells.

BLOOD: Blood is the most common sample tested, either as a liquid or as a dried stain. DNA is extracted from the white blood cells and is not present in either red blood cells or plasma.

Liquid blood samples are required from suspects and victims as knowns to be compared to the unknown samples recovered from the crime scene. Ideally, a minimum of 5 ml of blood should be taken, with a duplicate sample as well if that is possible. The samples should be collected in lavender [EDTA (Na_2) & EDTA (K_3)] or grey top (sodium fluoride) blood collection tubes. Using red (no preservative) or yellow top (ACD) tubes requires collection of a much greater amount of blood to obtain a sufficient quantity of DNA for testing. Tubes containing Sodium Heparin (green top) are the most difficult to work with and should be avoided.

Blood-stained material may also be used. Laboratory tests have shown that DNA can be extracted from very small stains, but clearly the larger the sample, the better the chance of success. The important factor is the concentration of a stain. As an example, we have been able to extract enough DNA from a stain a quarter of an inch in diameter but were unsuccessful in stains as large as 6 inches. Furthermore, the type of stained fabric can affect the extraction. Dark colored and heavily treated materials (e.g. blue jeans) have been found to complicate the extraction process more than light colored and light weight fabrics (e.g. cotton, nylon). **PRECUT STAINS ARE PREFERRED.** Blood stains made as standards by a crime lab are usually sufficient for the analysis.

SEMEN: Semen is the other commonly tested substance and is the main type of forensic evidence in sexual crimes. It is usually recovered on stained materials (e.g. clothing, bed sheets) or on vaginal, anal or oral swabs.

It is the sperm cell that actually contains DNA—not the seminal fluid. Therefore, before DNA analysis is performed, the recovered sample should first be tested to determine whether it contains sperm. Once sperm presence is confirmed, then the specimen can be used for DNA analysis.

As with blood-stained materials, the size of a semen stain needed for DNA testing depends on the-type of material and the concentration of the stain. Semen stains the size of half a dime (5 to 10 microliters) have been successfully examined. One or two swabs from a standard rape kit are usually enough to perform the **DNA FINGERPRINTING™** test, although quality DNA has been extracted from as little as one half of a cotton swab. Our laboratory experience has shown that semen samples that have been fixed to slides do not have a sufficient number of sperm cells for the analysis. Consequently, slides are not being accepted for testing.

HAIRS: Typically, a hair shaft that was cut or has fallen out naturally from someone's head will not contain a sufficient amount of DNA. It is the pulled or plucked hair root with the attached cells that is most appropriate for DNA analysis.

Different types of hair roots (head, moustache, beard, etc.) as well as different hair roots of the same type of hair (e.g. two head hair roots), may contain different amounts of DNA. Although we have been able to obtain enough DNA from as little as one hair root for analysis using single-locus probes, ideally, approximately 10 hair roots is the quantity required to successfully produce a DNA FINGERPRINT. We have also found that the mounting of hair on slides with Permount does not appear to influence the DNA banding pattern.

OTHER SAMPLES: In post mortem samples, the success of DNA analysis depends upon the state of decomposition of the body. This in turn depends upon the time since death and the temperature at which the body has been stored. Post mortem blood samples can be used only when the body is relatively fresh. Spleen or bone marrow are good sources of DNA. Another useful material is muscle, particularly from the thigh or upper arm. The size of these samples should be at least $1cm^3$.

In a case where the body is in a more advanced stage of decomposition, the best sample that might yield undegraded DNA is bone. Such a sample should be selected from spongy bones because they are the richest in DNA. (The best choice would be the rib.) A bone the size of 1cm × 1cm × 2–4mm should be sufficient.

Evidence Preservation

The evidence recovered from the crime scene should be stored properly to avoid DNA degradation. In general, all samples should be kept at a low temperature (4°C). If samples are not to be examined for several weeks, they should be frozen. The ideal temperature is -70°C. Repeated freezing and thawing should be avoided since this can reduce the amount of DNA which can be recovered. Before storage, stains, swabs and hair root samples should be thoroughly air-dried at room temperature to avoid the growth of mold and bacteria. Then they should be placed in moisture-resistant containers. Organs and tissues should be frozen without any added liquids or preservatives.

If freezing of a sample is not feasible, storing at room temperature is also acceptable. Paper bags are best for such storage. Plastic bags may trap humidity and cause sample deterioration. Plastic bags should only be used for storing samples which are to be frozen. Whenever they are used, it is vital that the air is completely removed and the bag sealed airtight to prevent condensation upon its removal from the freezer.

Fresh blood samples should be aliquoted, placed in plastic tubes and then frozen if they are to be stored for an extended period before testing. Making a stain from liquid blood, as is currently done in many cases, is another possibility. The stain should then be stored in the manner explained above.

Age Of Evidence

The successful extraction of DNA and the subsequent ability to achieve a result are related to the size, age and storage conditions of the sample. DNA can be found in specimens which are months or even years old. Dried-blood samples up to nine years of age have been successfully examined using the Jeffreys probes. Under some circumstances it may be possible to obtain results from samples of even greater age.

The age of a sample by itself is not a good indicator of whether DNA is undamaged and suitable for analysis. The condition of two samples of the same age may be drastically different. Diverse environmental factors, to which the samples have been exposed, cause this difference. Moisture in combination with high temperature is the most damaging element for the integrity of the DNA structure. For example, DNA in a wet blood stain will start to degrade in two days and in a semen stain will start to degrade within one week. The same samples kept dry at low temperature will stay intact for a much longer period of time.

Evidence Transportation

Samples should be shipped via an overnight carrier (preferable), or hand carried to the laboratory. The sender is responsible for following the chain of custody up to the time when the samples are transferred to Cellmark Diagnostics' care.

All samples that were stored frozen should be shipped on dry ice in styrofoam containers. Stains which were frozen do not need to be shipped on dry ice if packaged properly i.e. in airtight plastic bags. Dry samples, including swabs, cutout stains, etc., should be placed in paper envelopes.

Testing Procedure

All forensic samples are tested using single-locus probes.

TESTING TIME

Single-locus probes:

2 weeks – preparation (DNA extraction, digestion, electrophoresis, Southern blotting)
2 days – hybridization (probes)
1–3 weeks – developing

Multi-locus probes:

In cases where multi-locus probes are used, the membranes are stripped of single-locus probes and reprobed with the multi-locus probes. Developing time varies greatly with the amount of DNA present on the membrane (a smaller quantity of DNA on the membrane requires longer exposure).

Chain Of Custody Procedure

Cellmark Diagnostics' procedures guarantee a strict chain of custody. Upon receipt of the samples in the laboratory, the package is inspected, logged-in, and if stored, it is placed in a locked freezer. The technologist assigned to a case signs out any samples to be tested. A single chain-of-custody form accompanies all samples throughout the entire testing procedure. Anytime a sample goes from one station to another during the test, or is transferred from one tube to another, the procedure is witnessed by a second technologist and documented by both on the chain-of-custody form.

Court Acceptance Of DNA FINGERPRINTING™

DNA FINGERPRINTING™ became available in the United States on October 1, 1987, when Cellmark Diagnostics' U.S. laboratory began its commercial operation. Many forensic samples have been tested using this technique. Cellmark Diagnostics' court participation has been progressing rapidly. Expert testimony was given either at motion hearings or trials. In the instances where cases have gone to trial, the results of the **DNA FINGERPRINTING™** test were admitted into evidence. Further information will be provided when more cases have been adjudicated and/or an appellate level decision is reached. A current list of court cases where Cellmark Diagnostics' expert witnesses have given testimonies is available on request.

Examples of **DNA FINGERPRINTING™** use in the U.S. courts:

State of North Carolina
Case #: 87CRS5081 & 87CRS5080
Duplin County
Kenansville, North Carolina
Incest (Paternity)
Cellmark's expert witness testified on March 9, 1988. This was the first criminal case where the results of a *paternity* test using **DNA FINGERPRINTING™** were admitted into evidence. The suspect was convicted and sentenced in March 1988.

State of Florida
Case #: 87-1695-CF-M
Putnam County
7th Judicial District
Murder, Robbery, Sexual Battery
Cellmark's expert witness testified on March 23, 1988. The suspect was convicted of the crime. This was the first capital punishment case using DNA testing.

State of Wisconsin
Case #: 86PA145
Waukesha County
Circuit Court Family Division, Branch 10
Paternity Case
Cellmark's expert witness testified at a motion hearing to determine if DNA testing could be performed in a paternity action. The technology was accepted by the court. As a result, it was ordered that the **DNA FINGERPRINTING™** test be performed in this case of disputed paternity.

The **DNA FINGERPRINTING™** test, using the Jeffreys probes, has also been used in both criminal and paternity cases in the United Kingdom. It has been admitted as evidence by the British courts and is routinely used by the Home Office in their forensic work and in immigration cases.

A few examples of **DNA FINGERPRINTING™** use in the U.K.:

Ghana Immigration Case
A Ghanian boy, born in England, emigrated to Ghana to join his father. When he tried to rejoin his family in England, the authorities suspected that a substitution may have occurred and refused to admit him to the U.K. Using **DNA FINGERPRINTING™**, maternity and paternity were proven and the British authorities granted the boy's immigration.

Bristol Crown Court
Robert Melias was convicted of rape based on the **DNA FINGERPRINTING™** evidence presented in Bristol Crown Court. The DNA extracted from the semen stains on the victim's clothing was positively linked to Mr. Melias. He was sentenced to 8 years.

Leicester Crown Court
Two cases of rape/murder occurred in Leicestershire. **DNA FINGERPRINTING™** was used to first free a suspect wrongly accused in the case and then to positively identify the perpetrator. He was convicted and sentenced to life in prison.

10/88
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CELLMARK
DIAGNOSTICS

Manual

DNA FINGERPRINTING℠

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I. DNA FINGERPRINTING℠: THE FUTURE OF IDENTIFICATION

A. INTRODUCTION

Every year, in hundreds of thousands of court cases in the United States, a just decision rests on the ability of judges and juries to establish a person's identity. It happens in paternity suits and criminal cases. Who is the child's father? Is the drop of blood found at the scene of the crime from the defendant now on trial?

The evidence often conflicts and can carry the courts and the people whose lives they are affecting only so far. Often, judges and juries turn to technology for the answer - to a test capable of establishing identity or determining paternity beyond a reasonable doubt.

Until now, no test has been available that is both conclusive and simple to apply. Cellmark Diagnostics, a business unit of ICI Americas Inc., offers a revolutionary identification system called **DNA FINGERPRINTING℠**. This is the first "Yes/No" identification test because it provides positive identification. **DNA FINGERPRINTING℠** shows conclusively whether the genetic material in a drop of blood matches the genetic material obtained from the suspect or whether the DNA in a child's cells was inherited from the man or woman in question.

With facilities in Germantown, MD, and Abingdon, England, Cellmark Diagnostics is the first laboratory to provide the complete and confidential **DNA FINGERPRINTING℠** service commercially, using the unique probes developed by Professor Alec Jeffreys.

B. SOCIAL AND LEGAL APPLICATIONS

For the U.S. judicial and social services systems, **DNA FINGERPRINTING℠** offers a powerful new tool for applying justice quickly and fairly, at considerable savings in tax dollars and time spent. Every year, the abdication or denial of parental responsibility brings thousands of paternity cases to U.S. courts. This presents an enormous burden to state welfare budgets.

In thousands of cases of violent crime annually, suspects are not even arrested, often because evidence linking anyone to the crime is not strong enough. Solid evidence could lead to quicker arrests, prevent possible further crimes from being committed while the investigation proceeds, or verify a suspect's claim of innocence.

A large part of the problem in identifying individuals is the lack of a single, foolproof test. Conventional blood tests, for example, can determine who isn't the parent in a paternity case, but not who is. The more sophisticated HLA (Human Leukocyte Antigen) test has an exclusion capability above 90 percent, but that frequently is not enough. Additional tests are often ordered to reach the needed level of assurance required by the courts. The test results can take as long as two months to be reported, and the problem of court scheduling can stretch cases out for many more months.

In criminal cases, traditional chemical methods used for identification purposes can only test biological evidence of certain age and size. Their value is limited to being able to exclude a suspect but not to confirm a person's identity.

II. THE TECHNOLOGY

A. DNA (DEOXYRIBONUCLEIC ACID)

The science of DNA FINGERPRINTING℠ relies on the ability to look at an individual's basic genetic material - DNA. DNA, an organic substance found in the chromosomes in the nucleus of a cell, provides the genetic code which determines a person's individual characteristics (Figure 1). The code is expressed by the arrangement of four building blocks called bases. They are Adenine (A), Thymine (T), Guanine (G) and Cytosine (C). The bases are complementary: Adenine always bonds with Thymine and Guanine always pairs with Cytosine. These combinations are known as "base pairs" (Figure 2). Analogous to the letters of a four-letter alphabet, they are repeated millions of times in every cell and their order determines the unique characteristics of each individual. A short segment of this code might look like this:

-T-C-C-G-G-G-G-A-A-T-T-C-T-G-A-
-A-G-G-C-C-C-C-T-T-A-A-G-A-C-T-

Knowing the sequence of bases at any segment of one strand enables the scientist to specify the sequence of the opposite segment of the complementary strand.

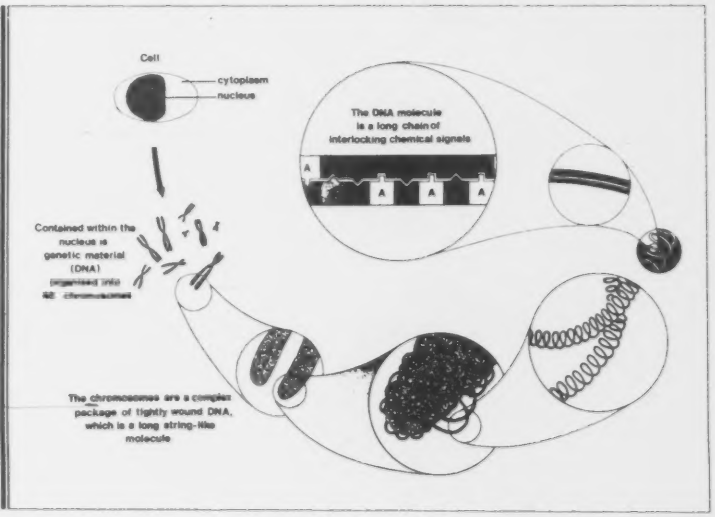
Physically, the DNA molecule resembles a ladder that has been twisted into a structure known as a "double helix". The sides of the DNA ladder are composed of alternating deoxyribose sugar and phosphate molecules. The bases form the rungs. Each rung is composed of one base pair (Figure 2).

The double helix can be separated into two single strands by heating the DNA or by chemical treatment which will cause the base pairs to break apart. When the DNA cools, the strands will reestablish their association in flawless alignment because of the complementary nature of the bases (Figure 3).

Only about 2-3 % of the approximately 3 billion base pairs in the DNA molecule code for genes. The function of the remaining "intergenic" sequences is unknown. It has been determined, however, that much of the intergenic DNA consists of sequences that tend to repeat many times throughout the molecule.

FIGURE 1

ORGANIZATION OF DNA IN CELLS



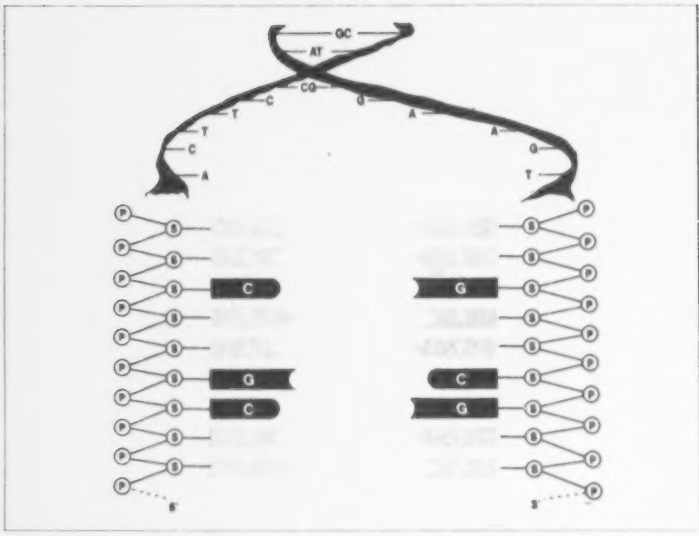


FIGURE 2

DNA MOLECULE

G - Guanine
C - Cytosine
A - Adenine
T - Thymine
P - Phosphate
S - Deoxyribose Sugar

Known as "repetitive DNA", these fragments represent breaks, or stutters, in the genetic code. For each person, their length and number are different. And these are the sequences that are utilized by DNA FINGERPRINTING℠ to genetically differentiate individuals.

B. DNA PROBES

The complementary nature of the DNA strands permits scientists to search for individual sequences (genetic characteristics) in DNA and detect them from the mass that results when the substance is extracted from millions of cells. Specific portions of the separated DNA strand are examined with the aid of restriction enzymes and DNA probes.

Restriction enzymes can be thought of as biological scissors that cut DNA into fragments. This segmentation is accomplished in a very specific and reproducible manner. Each enzyme recognizes a particular sequence in DNA and cuts the DNA molecule in places where this sequence is present. The number and size of the DNA pieces generated by a particular enzyme depend on where and how often the sequence recognized by the enzyme occurs in a particular person's DNA.

DNA probes are shorter fragments of a single-stranded DNA - perhaps several hundred or several thousand building blocks long - whose sequence of bases is known. These probes, when exposed to unknown DNA, seek out the sequence that they complement and attach themselves to it (Figure 3). Probes are made radioactive so the DNA sequences, to which they bind, can be detected. This is achieved by using an X-ray film on which the segments recognized by the radioactive DNA probe are visualized as a line or band.

To differentiate individuals, a restriction enzyme is used to cut out DNA fragments containing the previously mentioned "repetitive sequences". Differences in the lengths of the obtained pieces are then exploited. After sorting them by size by a process known as electrophoresis, those fragments which contain repetitive sequences are identified through the use of a Jeffreys DNA probe. Identified segments (bands) of different sizes appear in different locations on the piece of film and result in a band pattern similar to the UPC bar

FIGURE 3

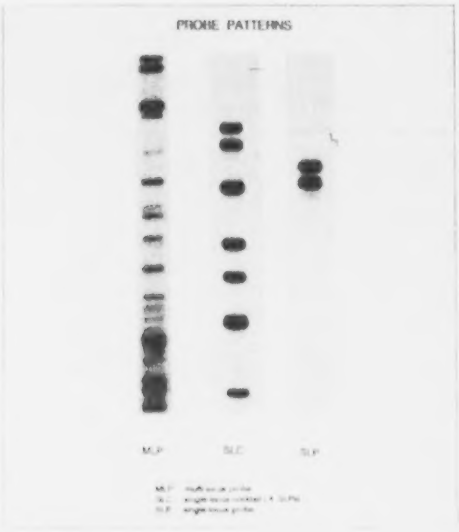


FIGURE 4

code that is used on grocery and other retail products. If the pattern of bands observed for one individual differs from the pattern observed for a second individual, then the first person has been genetically differentiated from the second. This genetically determined band pattern serves as the basis for identification.

Two types of probes are used for identification testing: one is referred to as a single-locus probe and the other as a multi-locus probe.

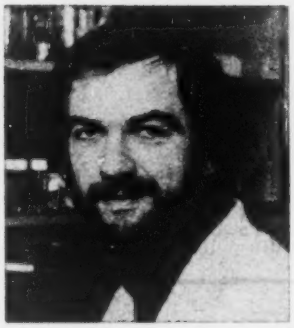
SINGLE-LOCUS PROBES (SLPs) detect a single segment of the repetitive DNA located at a specific site on a single chromosome. As such, use of the Jeffreys single-locus probe results in a DNA band pattern which contains at most two bands, one for each DNA segment recognized on each member of a chromosome pair (Figure 4). If the segments recognized on both chromosomes are the same size, only a one-band pattern is present on film. Thus, patterns generated by the SLPs are visualized as a one- or two-band bar code.

These one- or two-band patterns do not uniquely identify an individual. More than one person does exhibit the exact same pattern, because more than one person possesses the same detectable genetic characteristics seen by the single-locus probes. To obtain a more uncommon pattern of bands, several SLPs are used in the analysis.

Many single-locus probes are currently available. Their value for identification purposes depends upon how much variation can be detected by a particular probe in different people. To determine the discrimination rate, population statistics - that show what percentage of the population shares a particular band pattern - are applied. Due to their sensitivity SLPs are particularly useful for forensic samples.

MULTI-LOCUS PROBES (MLPs) detect multiple repetitive DNA segments located on many chromosomes. With one multi-locus probe a band pattern consisting of approximately 20 - 30 bands is obtained (Figure 4). Because these probes generate multi-band patterns, the chances of two people chosen at random matching at all the band positions is enormously low. In fact, the band patterns are (practically) unique for each individual. The chance of two unrelated people having the same DNA pattern detected by the MLPs is extremely low (on average 1 in 30 billion).

C. PROFESSOR JEFFREYS



The multi-locus probes were developed in the early 1980s by a British scientist, Professor Alec Jeffreys, at the University of Leicester while he was a Lister Institute Fellow. He was the first to recognize the enormous value of MLPs for identification testing and coined the term "DNA FINGERPRINTING".

For his contributions to genetics research, Professor Jeffreys has been named a Fellow of the prestigious Royal Society and has been awarded the Davy Medal by the Royal Society, the Carter Medal by the Clinical Genetics Society, and the Linnean Bicentenary Medal by the Linnean Society.

The Lister Institute of Preventive Medicine owns the rights to the technology (British Patent 2166445 and corresponding foreign patent applications), and Cellmark Diagnostics has the exclusive worldwide license to market this revolutionary technique.

When used for paternity establishment, the Jeffreys multi-locus probes are the only probes that allow one to determine the parentage of a child with certainty. They constitute the most accurate identification tool currently available.

For forensic applications, Professor Jeffreys has also developed several single-locus probes that he derived from the multi-locus probes. The combined use of the Jeffreys single- and multi-locus probes constitutes the most powerful existing identification technique for forensic testing.

The DNA FINGERPRINTING℠ test positively identifies individuals in cases involving paternity disputes, crimes, disasters and immigration. The technique is also employed to help understand genetically inherited diseases and to prove the authenticity and origin of human cell lines used in medical research.

Thousands of tests in the United States and Britain have proven the accuracy of the technique. The international scientific community has accepted its validity. Currently, more that than 600 laboratories around the world are conducting basic and applied research using the Jeffreys probes.

D. DNA FINGERPRINTING℠: THE LABORATORY PROCEDURE

Cellmark Diagnostics scientists extract and purify DNA from a small sample of blood, semen or other DNA-bearing cells. It is then cut apart with restriction enzymes at specific DNA sequences.

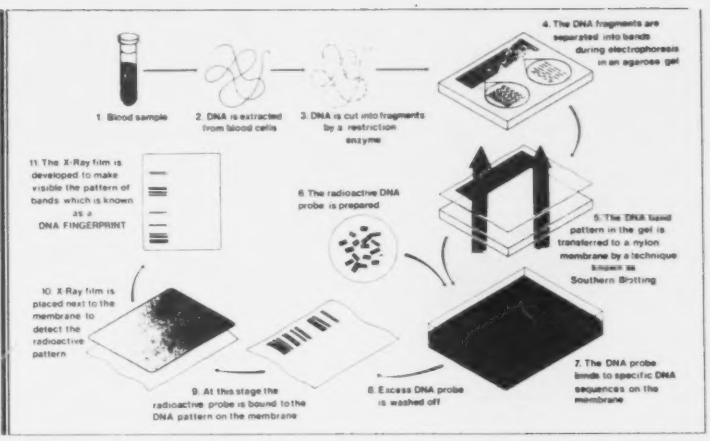
The obtained DNA fragments, which are negatively charged, are placed at one end of a sheet of gel and exposed to an electric field which causes them to move through the gel. The smaller pieces of DNA move more rapidly than the larger ones so the DNA becomes separated into bands by size - bands that are at this point invisible. The double-stranded fragments are then denatured (separated into single strands).

To preserve the obtained band pattern, scientists transfer the DNA fragments - which include the repetitive DNA as well as other sequences - from the gel onto a nylon membrane. Permanently affixed to the membrane, the DNA band pattern can then be subjected to further testing.

Next, the Jeffreys DNA probes, that have been labeled with a small amount of radioactive material, are added to a solution in which the membrane is immersed. The probes attach themselves to the specific invisible bands of repetitive DNA that they "fit". The DNA fragments containing these repetitive sequences are the only fragments recognized by the Jeffreys probes.

FIGURE 5

THE DNA FINGERPRINTING℠ PROCESS



To show where the probes have bound, a film that is sensitive to the radioactivity is placed against the nylon membrane. The probes expose the film allowing the scientists to obtain an image of the location of the repetitive DNA. This image is called a DNA FINGERPRINT or DNA band pattern.

The DNA FINGERPRINT on film looks like a bar code or a series of stripes. Each dark band present in the bar code occurs where the Jeffreys probe has unerringly located and zipped-up with its correct partner strand (repetitive sequence) among the millions of DNA fragments generated by the restriction enzymes.

III. APPLICATIONS

A. PATERNITY DETERMINATION

The pattern of bands generated using the multi-locus probes is as unique for each person as a normal fingerprint (except for genetically identical twins whose DNA is the same), but from which additional information relating to the parental origin can be derived. This is possible because approximately half of a child's DNA pattern is inherited from the mother and the other half from the father. This characteristic inheritance pattern is the basis for using DNA FINGERPRINTING℠ as a means of paternity determination. Although the child has DNA characteristics of both parents, the child is still a unique individual.

Cellmark Diagnostics uses two Jeffreys multi-locus probes (33.6 and 33.15) for paternity testing. On average, these two probes together can detect between 40 and 60 different DNA segments in a given individual. Thus, 40 to 60 different bands are visualized on film and can be used as points of comparison.

To establish paternity, DNA FINGERPRINTS of the mother, child and the alleged father are compared. All the bands that are in the same place in the child's and mother's band patterns are identified and marked. The remaining bands in the child's DNA FINGERPRINT must have come from the biological father (Figure 6 & 7).

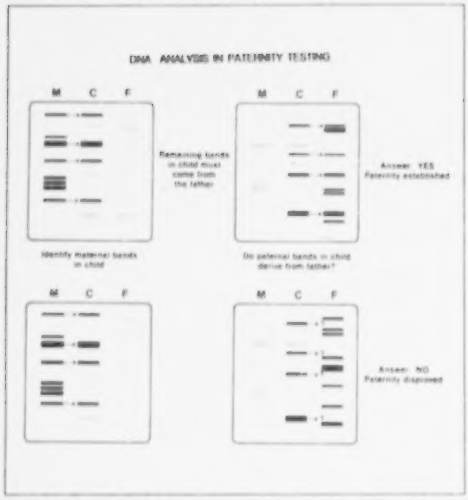


FIGURE 6

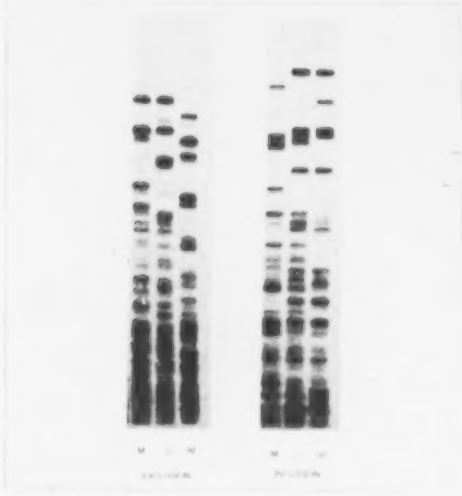


FIGURE 7

In the interpretation of the results, the number of paternal bands that are present in the putative father is assessed. Then, the probability of another person having that exact number of bands in those exact positions is calculated. There is a 25% chance that two unrelated individuals will share a given band (Nature (1985) 314: 67-73, Nature (1985) 316: 76-79). The table below illustrates that the more bands that are present in the pattern, the lower the probability of another person having all of those bands.

NUMBER OF BANDS	PROBABILITY OF AN UNRELATED INDIVIDUAL HAVING THE SAME BANDS
1	1 in 4
2	1 in 16
4	1 in 250
6	1 in 4,000
8	1 in 65,000
10	1 in 1,000,000
12	1 in 17,000,000
14	1 in 268,000,000
16	1 in 4,300,000,000
18	1 in 68,000,000,000
20	1 in 1,000,000,000,000

Mutations may occur in the inheritance of band patterns generated by the Jeffreys probes. They result in one or two (and in extremely rare instances three) bands that cannot be assigned to either the mother or the father. These unassigned bands can be expected in cases of true paternity. This is because the multi-locus probes detect segments at which DNA deletions and additions may occur in the transmission of the DNA code from generation to generation.

The statistics that are employed conservatively assume that all unassigned bands are paternal. In some cases, maternal mutations can be detected by using the Jeffreys single-locus probes which identify sequences where mutations occur. These bands are therefore eliminated from consideration as paternal specific bands.

The presence of unassigned bands impacts on the probabilities calculated. For example, in one case 14 non-maternal bands in a child were all found in the alleged father. The probability of an unrelated man having all of these bands is 1 in 268,000,000. In another child, 14 of 15 non-maternal bands were found in the alleged father. The probability of an unrelated man having 14 of the 15 bands is 1 in 23,000,000. The higher probability of a random match in the latter case results because there is more chance of randomly choosing 14 of 15 items than there is of choosing 14 out of 14.

Multi-locus probes minimize the effect that mutations may have on the final result due to the large number of bands which are present in the pattern. The use of two different multi-locus probes even further reduces the possibility of making a false determination. Mutation rates for the Jeffreys multi-locus probes were reported in Nature (1987) 332, 278-281. When a man is not the true father of a child, the majority of the paternal bands in the child's DNA band pattern will not match the DNA band pattern of the alleged father. There can be no confusing a mutation with a false exclusion when using the DNA FINGERPRINTING℠ test.

For paternity applications, the odds that two unrelated people possess the same DNA band pattern have been calculated to be, on average, 30 billion to 1. Given that the Earth's population is about 5 billion people (only 2.5 billion males) it is impossible to be more sure of a paternity determination with any other available test.

DNA FINGERPRINTING℠ has been used to establish paternity even in instances where the putative father is deceased. In cases where an inheritance or social security benefits are at stake, it may be beneficial to pursue paternity determination. To perform the test, adequately preserved samples from the autopsy can be used. If the samples are not available, in certain cases, the alleged father's DNA FINGERPRINT can be reconstructed by using samples from other close relatives. In such cases, the degree of certainty will depend upon which relatives are available. For example, samples from both parents of the alleged father would allow one to determine paternity with practically the same certainty as would be possible if that person's DNA sample were available.

Band patterns generated by single-locus probes are also inherited in Mendelian fashion (half from the mother and the other half from the father). However, single-locus probes are not being used for paternity testing as an exclusive test because the obtained patterns are not individual specific and paternity cannot be determined to the same degree of certainty as with the multi-locus probes.

Other paternity testing laboratories use single-locus probes as an "add-on" test to traditional testing procedures. The obtained probability of paternity is not nearly as definitive as that of the DNA FINGERPRINTING℠ test. Mutations can also alter the expected band patterns detected by SLPs in offspring. Since there is only one point of comparison between each parent and a child with each SLP, mutation of this one point can cause false interpretation of the results.

B. FORENSIC TESTING

DNA FINGERPRINTING℠ is being used to conclusively link a biological sample recovered from a crime scene to one obtained from a suspect. The test helps to exclude falsely accused suspects or to pinpoint the perpetrator during the investigative phase of a criminal case. When used as a trial tool, it strengthens the evidence that may be presented to the court. The test can also be used to determine the identity of the victim in cases where no other form of identification is possible.

Analysis of DNA patterns in a criminal case is a comparative technique. The DNA band pattern generated from a specimen recovered at the scene of a crime is compared to the DNA band pattern generated from a sample provided by the suspect. If the two patterns match exactly, then it is virtually certain that the accused left that specimen at the crime scene (Figure 8 & 9).

In forensic applications, the Jeffreys single-locus probes are used for identification of the specimens.

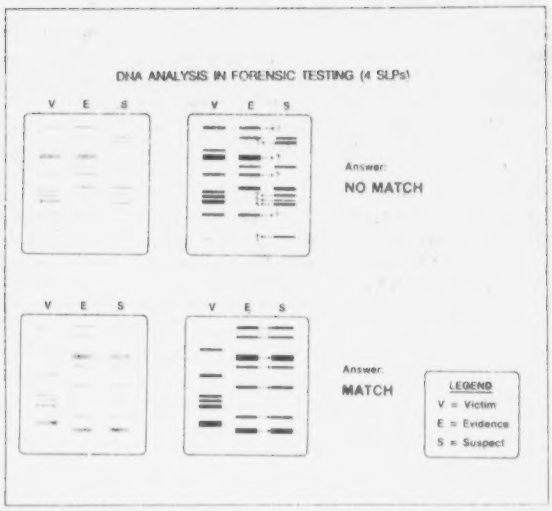


FIGURE 8

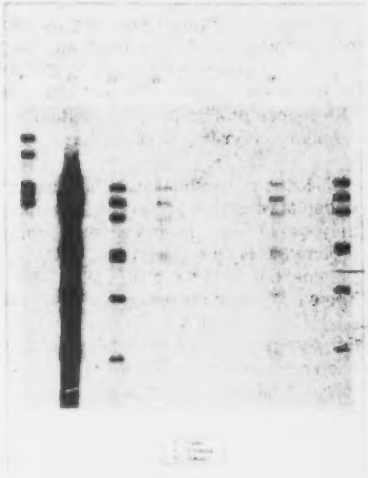


FIGURE 9

Although in most cases not as discriminating as the multi-locus probes, the single-locus probes can differentiate individuals to the degree of excluding the world's population. They also offer other advantages. Frequently, forensic evidence contains amounts of DNA so small that the multi-locus probes cannot be used. Single-locus probes are about 10 times more sensitive and allow Cellmark scientists to obtain a DNA band pattern from as little as 20-50 nanograms of sample DNA. A blood stain or semen stain the size of a head of a pin, or a single hair root, may be all that is needed to make an identification.

Another major benefit of the Jeffreys single-locus probes is their ability to resolve mixed stains. This property is especially relevant to the examination of mixed-semen samples from a multiple-assailant rape case, or mixed-blood samples from a homicide scene. In such cases, the mixture could be analyzed first with one single-locus probe to determine how many individuals were involved. An example of such an analysis is shown in Figure 10. Because each probe produces a pattern of at most two bands per person,

FIGURE 10

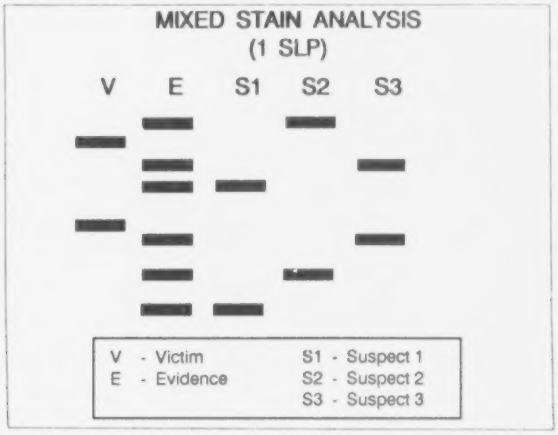
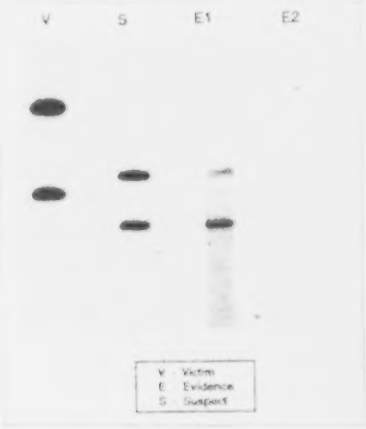


FIGURE 11



a six band pattern obtained from the mixture indicates that it contains samples from at least three individuals. Subsequent matching of the suspects' band patterns with those in the mixture would confirm their involvement. A combination of four single-locus probes, used sequentially or as a "cocktail", would further increase the accuracy of the identifications.

Not only are the Jeffreys single-locus probes at least as discriminating as any single-locus probes currently available, they can be used to cross reference bands present in the DNA patterns of suspects prepared using the Jeffreys multi-locus probes. No other company has this capability. Combined use of both types of Jeffreys probes represents the current optimum in identification testing for forensic applications.

DNA FINGERPRINTING℠ can be applied to several areas of forensic work. In rape cases, semen containing sperm can be used to positively identify suspects. This can be accomplished even if the semen sample is mixed with blood or other fluids from the victim. In homicide cases, even small pieces of the assailant's tissue, if available, could be analyzed for comparison with the DNA from suspects to positively identify the culprit.

Assuming the availability of first order relatives or existing samples containing DNA, the DNA FINGERPRINTING℠ technique also might allow the identification of crash, flood and fire victims. Small amounts of bone or tissue would be sufficient to obtain DNA FINGERPRINTS.

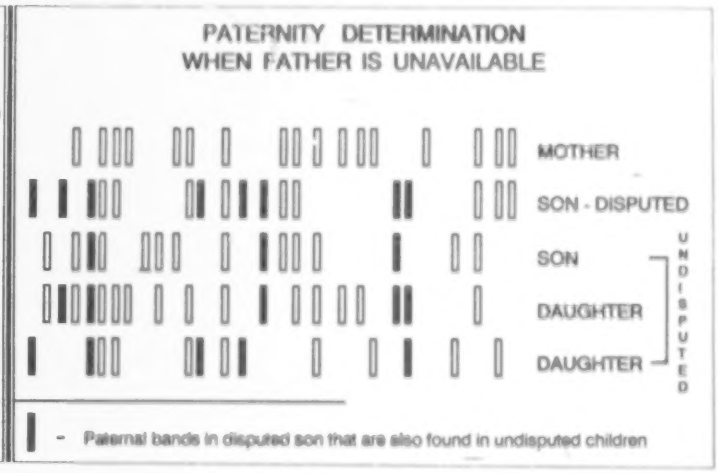
Unlike paternity test results, mutations do not affect the results of forensic comparisons when using single-locus probes. This is due to the fact that mutations only cause a problem when the genetic material is passed down from parents to offspring and DNA comparisons are being made across generations. The majority of forensic testing involves examining DNA samples from the same individual and any detectable mutations will be present in every cell within the that individual.

C. IMMIGRATION CASES

DNA FINGERPRINTING℠ is applicable in immigration cases involving persons who seek entry into the United States on the grounds that they are blood relatives of American citizens. If there is a reason to doubt the authenticity of these claims or the documents proving the lineage, the questioned relationship can be verified using the Jeffreys multi-locus probes.

FIGURE 12

EXAMPLE OF IMMIGRATION TESTING



Paternity or maternity is determined by testing both parents and the child. However, parentage can also be determined in situations where one of the parents is not available for testing. In such cases, the DNA band pattern of that person is reconstructed by analyzing DNA of other blood relatives (siblings, parents). The certainty of the results will depend on the circumstances of each case (e.g. which and how many members of the family are available for testing).

An example of a case where the alleged father is unavailable for testing is shown in Figure 12. First, the children's DNA patterns are compared to their mother's to identify the maternal bands. The remaining bands must have been inherited from the father. The paternal bands in the undisputed children are then compared to the paternal bands in the child in question. If they are unrelated, their band sharing would be 25% or less. However, if the band sharing is higher than that, the certainty of the result (their father being also the biological father of the child in question) will depend upon how many paternal bands in that child are also present in the undisputed children.

DNA FINGERPRINTING℠ can also provide valuable information regarding other biological relationships between close relatives. By looking at the number of bands shared by two individuals, it can be established whether they are related.

D. OTHER USES

There are other applications where the DNA FINGERPRINTING℠ test can prove to be valuable.

Cell line authentication. The test is employed to check for cross-contamination by other cell-lines, which is extremely important to scientific researchers, pharmaceutical and biotechnology companies.

Animal applications. DNA FINGERPRINTING℠ is useful in pedigree determination of dogs, fish, birds and other animals.

IV. APPENDICES

A. CORPORATE BACKGROUND

Cellmark Diagnostics offers a complete and confidential DNA FINGERPRINTING℠ service. DNA FINGERPRINTING℠, the unique discovery of Professor Alec Jeffreys, permits positive identification of individuals, using their genetic structure.

Formed in 1987, Cellmark Diagnostics U.S. is a business unit of ICI Americas Inc. ICI Americas Inc. is the U.S. operating subsidiary of Imperial Chemical Industries PLC, a Britain-based multinational chemical and pharmaceutical company. ICI, with worldwide sales exceeding $20 billion, is one of the world's leading companies involved in healthcare and biotechnology.

Cellmark Diagnostics draws from the resources and expertise provided from ICI Diagnostics, a special unit established in 1984 within the corporation's healthcare portfolio. ICI Diagnostics engages a team of dedicated researchers devoted solely to important and practical applications of gene probe technology and genetic testing.

Cellmark Diagnostics also takes advantage of the parent company's tremendous research and development capabilities. In 1988, ICI allocated almost $2 million a day to R&D, with more than 9,000 scientists devoted primarily to biological research. A significant amount of research monies is dedicated to molecular biology and gene probe development.

Cellmark Diagnostics' Germantown, MD, laboratory employs molecular biologists, geneticists, chemists and medical technologists who conduct the DNA FINGERPRINTING℠ testing in a state-of-the-art facility.

Cellmark Diagnostics' DNA FINGERPRINTING℠ service exemplifies ICI's reputation for innovation and discovery and its commitment to remaining a World Class company at the leading edge of technological advancement.

B. GLOSSARY

ALLELE
One of several alternate forms of a gene occupying a given place on the chromosome.

BASES
The chemical units (adenine, thymine, guanine and cytosine) in DNA molecules that constitute the genetic code.

CHROMOSOME
The location of hereditary (genetic) material within the cell. Genes are found arranged in a linear sequence along chromosomes.

DNA
Abbreviation for deoxyribonucleic acid. The molecules containing hereditary (genetic) information.

ELECTROPHORESIS
A technique for arranging biological particles by exposing them to an electrical charge. In DNA electrophoresis the distance a particle travels on the electrophoretic field depends on its size; smaller particles will migrate farther than larger particles.

GENE
The portion of a DNA molecule that comprises the basic functional hereditary unit; a segment of DNA that codes for the production of a specific protein.

GENETIC MARKER
Any character that acts as a signpost or signal of the presence or location of a gene or hereditary characteristic in an individual in a population.

KILOBASE (Kb)
A unit of 1,000 bases in DNA.

LOCUS
The position on a chromosome at which the gene for a particular trait resides.

NUCLEOTIDES
The fundamental units of nucleic acids. They consist of one of the four bases (adenine, guanine, cytosine and thymine), a sugar and a phosphate unit.

POLYMORPHISM
A naturally occurring or induced variation in the sequence of genetic information on a segment of DNA.

PROBE
A fragment of DNA carrying the complementary code for a specific base sequence. Probes can be used to detect variations in base sequence that establish genetic identity.

PROTEIN
A molecule consisting of linked amino acids. Proteins are the products of gene expression and are the functional and structural components of cells.

RESTRICTION ENZYME
Enzymes that act as molecular scissors to cut DNA molecules at specific locations.

RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP)
A polymorphism in DNA sequence that can be detected on the basis of differences in the length of DNA fragments produced by cutting DNA with a specific restriction enzyme.

SOUTHERN BLOT
A technique for transferring DNA fragments separated by electrophoresis to a special filter on which specific DNA fragments can then be detected by radioactive probes.

TANDEM REPEATS
Multiple copies of the same DNA sequence arranged in direct succession along a chromosome.

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The FAMILY LAW REPORTER®

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NEWS NOTES

LEGITIMACY & PATERNITY — IDENTIFICATION — "DNA FINGERPRINTING"

A new, precise method of personal identification, with significant implications for criminal and paternity cases, made its American debut last week in Washington, D.C. The process, known as "DNA Fingerprinting," was announced Cellmark Diagnostics, a Germantown, Md., biotechnical concern.

According to Cellmark president Robert H. Gottheiner, the process can be used to determine paternity of a child "without doubt," and is the first "yes-no" test of paternity that has been developed. Unlike HLA testing, which operates to exclude a certain percentage of the population from the group of persons who could be identified as the father of a particular individual (see Monograph at 9 FLR 4009), the DNA fingerprinting process is claimed to match to an absolute certainty the DNA fingerprint of a child with his or her mother and father.

The technique was developed by British researcher Dr. Alec Jeffreys out of his work with DNA probes, which serve to isolate particular portions of the DNA chain, and which have a wide application of uses. In every person's DNA chain there are breaks (called "stutters") that appear in a repetitive pattern throughout the DNA molecule. Jeffreys discovered in the early 1980s that he could isolate portions of the molecule that contain the stutters.

The DNA fingerprinting method involves the extraction of DNA from a small sample of blood, semen, or other DNA-bearing cells, which is then cut apart with an enzyme that fragments the DNA double strands at specific DNA sequences. Then a series of procedures further separates the DNA fragments and transfers them to a nylon membrane where the probes are added. The probes attach themselves to specific invisible bands of repetitive DNA that they "fit"—like the teeth of a zipper. Then the membrane is exposed to an X-ray film resulting in a "DNA fingerprint," which on film resembles the UPC bar code used on grocery and other retail products.

The bands of the fingerprints are analyzed and compared to establish a positive identification. Each individual's bar code is unique, except in the case of identical twins. According to Jeffreys, the chances that two unrelated individuals will have the same DNA fingerprint are one in a quadrillion. Even with siblings, the chances of identical DNA fingerprints (except with identical twins) are one in 10 trillion. For paternity tests, the examiner takes a sample from the mother, the putative father, and the child, and obtains a DNA fingerprint from each sample. According to Jeffreys, every bar in the child's code can be attributed to either the mother or the father, with an even split in the number attributable to each. Thus the first step in the analysis is to compare the child's fingerprint with the mother's, and locate all of the bars that match the mother's code. The remaining bars in the child's fingerprint then can be compared with the putative father's. If the man is, in fact, the father, every remaining bar will match up with one from the father's fingerprint. If the man is not the father, very few of the bars will match. The process has already been effectively used in cases in the United Kingdom. □

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TRIAL PRACTICE SERIES

BNA CRIMINAL PRACTICE MANUAL

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CURRENT REPORTS

DNA FINGERPRINTING ID METHOD MAY STREAMLINE INVESTIGATIONS

A British researcher asserts that the innovative identification process of "DNA Fingerprinting" could radically change the handling of any case in which identity of a particular person is at issue. Dr. Alec Jeffreys, who developed the process in England, says that the chances of two people, other than identical twins, having the same DNA fingerprint are astronomical. Thus in cases of disputed paternity, or criminal investigations involving physical evidence such as blood, semen or hair, investigators should be able to match "DNA fingerprints" from the samples to a degree of certainty far beyond that possible with existing methods. The process has been accepted by British courts in both civil and criminal matters

DNA "FINGERPRINTING" ADVANCED AS IDENTIFICATION TECHNIQUE

Imagine a paternity test which could determine to an absolute certainty that a particular man is or is not the father of a particular child. Imagine that this same test could definitively establish the identity of the person who leaves traces of semen, blood, hair, or other tissue at the scene of a crime. Such a test may no longer be solely a product of imagination, according to Dr. Alec Jeffreys, a British researcher, who recently came to Washington, D.C., to launch the marketing and development of the test in the American market.

Labeling the process a "true scientific breakthrough," Robert H. Gottheiner, president of Cellmark Diagnostics in Germantown, Md., announced what he called the American debut of the process, called "DNA Fingerprinting."

According to Gottheiner, paternity can be determined "without doubt" with the use of the DNA fingerprinting process, which is the first "yes-no" test of paternity that has been developed. The Human Leukocyte Antigen (HLA) test operates to exclude a certain percentage of the population from the group which could be identified as the father of a particular individual. The HLA test does not, and cannot, identify any particular person as the father. By contrast, the DNA fingerprinting process is claimed to match to an absolute certainty the DNA fingerprint of a child with his or her mother and father. In the forensics field, the test is said to provide a positive identification of a known sample from a suspect to tissue traces left at the scene of the crime.

The process presented at a press conference in Washington on Sept. 17 is the brainchild of British researcher Dr. Alec Jeffreys, who has been working in the field since the early 1980s. Cellmark Diagnostics, which is a subsidiary of ICI Diagnostics, a branch of the British corporation ICI, came to Germantown earlier in 1987 and set up the first of several diagnostic laboratories planned for the United States.

What Is a DNA Fingerprint?

Dr. Jeffreys' method came out of his work with DNA probes, which serve to isolate particular portions of the DNA chain, and which have a wide application of uses. In everyone's DNA chain there are breaks (called "stutters") which appear in a repetitive pattern throughout the DNA molecule. Jeffreys discovered in the early 1980s that he could isolate portions of the molecule which contain the stutters.

The Cellmark Diagnostics presentation explained the process as follows: After a laboratory process that extracts and purifies DNA from a small sample of blood, semen or other DNA bearing cells, the DNA is cut apart with a restriction enzyme that fragments the DNA double strands at specific DNA sequences. Then a series of procedures further separate the DNA fragments and transfer them to a nylon membrane where the probes are added. The probes attach themselves to specific invisible bands of repetitive DNA that they "fit" — like the teeth of a zipper. Then the membrane is exposed to an x-ray film resulting in a "DNA Fingerprint," that on film looks like a bar code, or a series of stripes that are used on grocery and other retail products. The bands of the fingerprints are analyzed and compared to establish a positive identification.

The bar code which results from the analysis is unique to every individual except identical twins. Because the DNA of identical twins is the same, the DNA fingerprint of twins will always be a perfect match. According to Jeffreys, the chances that two unrelated individuals will have the same DNA fingerprint are 1 in 1,000,000,000,000,000. Even with siblings, the chances of identical DNA fingerprints (except with identical twins) are 1 in 10,000,000,000,000.

For paternity tests, the examiner takes a sample from the mother, the putative father and the child, and obtains a DNA fingerprint from each sample. According to Jeffreys, every bar in the child's code can be attributed to either the mother or the father, with an even split in the number attributable to each. Thus the first step in the analysis is to compare the child's fingerprint with the mother's, and locate all of the bars which match the mother's code. The remaining bars in the child's fingerprint then can be compared with the putative father's. If the man is, in fact, the father, every remaining bar will match up with one from the father's DNA fingerprint. If the man is not the father, very few of the bars will match.

In the forensic setting, the examiner does a direct comparison between the sample taken from the crime scene, and a sample obtained from a suspect. Again, there either will be a direct match or there will be virtually no similarity between the two samples.

Advantages to the New Technique

Jeffreys explained that as an identification tool, the DNA fingerprint is superior to other methods of identification available today in a number of respects. First, Jeffreys' empirical evidence shows that either the bar codes match exactly, or only a few points in the pattern match. Jeffreys has not found a situation where, for example, only two or three points don't match. Thus a person analyzing the results of a DNA fingerprint does not have to make a subjective judgment about how much dissimilarity can be tolerated before the two samples are determined not to match.

In the traditional fingerprint situation, even fingerprints which an expert has declared as a match will have points of similarity and dissimilarity, and the examiner has to make a subjective judgment as to when a dissimilarity is sufficiently explained so as not to affect the reliability of the match. Two different fingerprint examiners therefore might have different opinions about the same set of fingerprints.

In contrast, two DNA fingerprints from the same person will always be an identical match, except where a mutant gene is present. In those cases, one bar of the code will be dissimilar, but an examination of another portion of the chain will reveal a perfect match. According to Jeffreys, DNA fingerprinting analysis as done in his laboratory will always involve the examination of two separate sequences of DNA, so the second test serves as a back-up if a mutant gene is part of the first test.

Another advantage to the DNA fingerprinting technique is that it requires a small amount of tissue in order to perform the analysis. One or two drops of blood, 10 hair roots, or a trace of semen will suffice for the test, and the samples can be much older than those needed for other tests, for example, traditional blood typing and analysis. The DNA molecule is very stable, noted

Jeffreys, and therefore will still be available to the researcher in a sample which is weeks, months, and even years old. The state of the art in traditional blood grouping tests has improved so that older samples can be used in such tests, said Jeffreys, but the older the sample the more likely it is that you will get ambiguous results, or results that are not reliable.

The testing process also takes less time than other methods of identification, according to Gottheiner, Cellmark's president. In the United States, paternity testing can take up to 30 days, depending on how busy the lab is, and only rarely can be done in two weeks. Forensic identification techniques take even longer, usually between one to six months. The DNA fingerprint, on the other hand, takes only a week or two to develop, and thus comparisons can be made much more quickly than in the past. The cost of the process also is comparable to traditional methods, said Gottheiner, who mentioned a ballpark figure of between $500 and $800 for the comparison.

The British Experience

The Jeffreys method has been used in a number of cases in Great Britain, and has been accepted in both civil and criminal courts. Two recent cases discussed by Jeffreys illustrate the potential uses of the process in widely different settings. In the first case, a young girl's body was discovered and it was determined that she had been raped and strangled. Soon afterward, the police arrested a 17-year-old youth in connection with that murder and another, similar crime from several years earlier. The Jeffreys' method was used to compare tissue samples from the two crimes and it was determined that the same individual committed both rapes. The investigators went one step further and analyzed the DNA fingerprint of the person who had been arrested for the crimes. The DNA analysis convinced the authorities that the suspect was not the person who raped the young women, and he was released.

In another case, the immigration authorities used the process to determine the parentage of a Ghanian boy born in England, who had emigrated to Ghana to live with his father. When he tried to reenter the United Kingdom, the immigration authorities suspected that a substitution had occurred, and refused to admit him. Although the father was not available to be tested, DNA fingerprints were taken from the boy, his alleged mother, brother, and two sisters. Using fragments present in one of the siblings but absent from the mother, the researchers reconstructed a fingerprint of the father. The scientists then compared the DNA fingerprints of the boy, his father, and his mother and found that half the fragments in the boy's pattern were present in the mother's code, while the remainder appeared in the father's. Thus, maternity was proved to the satisfaction of the British authorities and the boy was allowed to rejoin his family.

Jeffreys also spoke of a case which he may handle in Britain involving a rape victim who discovered that she was pregnant and wants to know the identity of the father. If the woman knows that her husband is the child's father she will carry it to term, but if she became pregnant as a result of the rape she may decide to terminate the pregnancy. According to Jeffreys, the DNA fingerprinting process can be done on a small amount of what will become placental matter as early as 8 to 10 weeks after conception. Although the police have no suspects in the rape case as yet, the DNA fingerprint of the developing fetus can be compared with the rape victim's husband, and if there is not a match it can be concluded that the rapist is the father of the child.

Another, more off-beat use to which the Jeffreys method has been put is in paternity testing of a dog. Every living organism contains DNA, explained Jeffreys, and there is no differentiation in type of DNA between genders, or even among species. Thus the same type of DNA fingerprint that can be obtained from humans can be obtained from dogs, cats, cattle, and even plants. In the area of animal breeding, the test can be used to settle disputes about the bloodlines of a particular pedigreed animal, and Jeffreys was called upon by a dog breeder to perform just such a test.

Legal Implications of Process

Also appearing at the press conference was James E. Starrs, who is a professor of law and forensic sciences at George Washington University, and is a consultant to Cellmark Diagnostics. Starrs predicted that the DNA fingerprint method will "put an end to microscopic hair analysis," and that we could see a system of law enforcement in which traditional fingerprint files would be supplemented with DNA fingerprint files. We allow the police to take fingerprints routinely upon arrest, noted Starrs, and it could be that at some point taking a hair sample, for example, in order to develop a DNA fingerprint of a criminal suspect will become a matter of course.

Starrs expressed the opinion that the DNA fingerprinting method poses some constitutional concerns, but that those concerns are not much different than those posed by existing methods of identification, and that the test offers enormous gains in investigation.

Starrs posed the hypothetical problem of a rape case, in which the victim has been murdered and the police obtain a mixed sample of semen and vaginal fluid. Testing according to the ABO blood group system indicates the presence of the A blood group in the stain. Whether the presence of this blood group is from the vaginal fluid or the semen is unknown and, at present, scientifically unknowable. The A antigen reaction could have come from bacterial contamination of the stain, and in fact tests reveal that the victim is of the A blood group and is a secretor. This latter fact means that the victim's A antigen will appear in all of her bodily fluids and could have accounted for the test results.

Carrying the hypothetical further, Starrs posited the existence of a suspect, arrived at through other methods of investigation. The police obviously want to determine whether he was the donor of the semen found in the victim. According to Starrs, our present method of going about such a comparison is "convoluted and tortured in its performance and uncertain in its results." The police will seek the answers to two questions, said Starrs. First, is the suspect a secretor, and therefore a person whose other bodily fluids will contain antigens of his blood type? If so, is the ABO blood group of the suspect the same as that identified in the mixed stains from the victim?

Obviously, the questions can best be resolved by obtaining a semen or blood sample from the defendant and performing the necessary comparisons. Under the Fourth Amendment, however, a search warrant is needed to obtain either blood or semen from the suspect. Depending on the amount of evidence against the suspect, the police may or may not be able to establish probable cause sufficient to justify the issuance of the warrant. In addition, due process concerns arise in an attempt to obtain a semen sample from an unwilling suspect, as the procedure to obtain the sample is highly intrusive. A warrant is not necessary to obtain a saliva sample, but scientific conclusions drawn from saliva, because of aberrant reactions and the like, are not necessarily transferable to semen.

Even assuming that the constitutional roadblocks are removed, the results of the ABO blood grouping analysis may not bring the police any closer to identifying the culprit. If, for example, the suspect is blood group A, the presence of the A antigen could have come from either the victim or the suspect. Even if the suspect has a different blood type, if he is not a secretor then the victim's A antigen could mask the presence of the suspect's blood type in the sample. In short, the state of the art in blood grouping is such that in many cases the analysis is inconclusive.

DNA analysis, on the other hand, will enable us to distinguish the DNA types within the mixed stain, something no current methodology can lay claim to with any assurance, according to Starrs. The process also circumvents the constitutional problem inherent in attempting to compel the suspect to provide either a blood or semen sample. The removal of as few as 10 hairs from the suspect's head will suffice to perform the DNA analysis, and this procedure arguably is minimally more intrusive than that which is used to obtain fingerprints, voiceprints, or a handwriting sample.

Nevertheless, Starrs cautions restraint in seeking to use the results of the DNA fingerprinting process in a court of law. The process is new, and as yet has not undergone the kind of extensive field validation work which Starrs feels is necessary for its acceptance under either the *Frye* [*Frye v. U.S.*, 293 F 1013 (CADC 1923)] test or the more relaxed relevancy standard of the Federal Rules of Evidence.

Starrs is confident that continued validation work will simply confirm the accuracy and reliability of the process, but noted that a premature effort to gain acceptance by the courts could be detrimental in the long run. Once a process has been rejected by a state, particularly a major jurisdiction like California or Michigan, there often is a snowball effect and other courts will follow the early lead. If that is the case, then even new evidence of the test's acceptance in the scientific community might not change a judicial hesitancy to allow the evidence into a trial.

In its present stage of development, the DNA fingerprinting process may be subject to challenge, according to Starrs. For one thing electrophoresis, which is a big part of the process, has not been universally accept-

ed in American courts. Also, field studies are needed, as for the most part the testing has occurred under laboratory conditions. In Starrs' view, the DNA fingerprinting process will have its biggest impact as a forensic tool. Starrs noted that at present many courts and some legislatures have specifically accepted the HLA test as reliable in paternity suits, and there may not be a desire or a need to turn to another method for making such determinations. The courts have not made that same judgment about the reliability of existing ABO blood grouping tests, however, and thus the forensic field is more open.

Even if the test is not immediately accepted in the courts, it would appear that the process even now provides a valuable investigatory tool. It is common practice in some places, for example, to take a polygraph of a suspect or a key witness. The polygraph results themselves generally would not be admissible in court, but the police will sometimes rely on them in directing their investigation, or in making charging decisions.

Similarly, a rape suspect may offer to submit to DNA fingerprinting in order to establish unequivocally that he is not guilty of the crime. Presumably, a favorable test result will remove suspicion from the individual. The opposite result, on the other hand, may lead to a negotiated plea in much the same way that strong fingerprint evidence will sometimes lead to a plea. In any event, the DNA fingerprint method portends vast changes in the legal community in terms of proof of identification.

Crim.L.R.

Method and Applications of DNA Fingerprinting: A Guide for the Non-Scientist

By K. F. Kelly*, J. J. Rankin† and R. C. Wink‡

The development of a new technique, known as "DNA fingerprinting," has enormous potential for use in medical science and forensic investigation. In this article the scientific background to this technique is described in terms which, it is hoped, will be comprehensible to the non-scientist and which will make clear its forensic and legal importance. The accuracy of identifications made using DNA fingerprinting is discussed and particular areas of possible application are indicated.

Existing investigative techniques such as blood group analysis or immunological tests have both qualitative and quantitative drawbacks compared to DNA fingerprinting. A putative father in a paternity case, for example, can be excluded on the basis of blood group analysis but can be positively identified only in exceptional circumstances. Immunological techniques have many applications in forensic science, including identification of blood components and the specific origin of tissues, but these methods depend for their accuracy on the properties of the sample such as age, purity and the quantity available for analysis.[1]

The strength of the DNA technique rests in the intrinsic properties of Deoxyribonucleic Acid (DNA), the material of which chromosomes are made. Only tiny quantities are required for analysis, and each individual's DNA is unique. Under the correct circumstances DNA is remarkably stable and indeed has been extracted from Egyptian mummies.

The new technology has so far not been applied in any court case in the United Kingdom, North America or elsewhere so far as is known. It has, however, been utilised in one immigration dispute to establish a mother–son relationship, and has been instrumental in securing the release of a youth charged with murder. It is reported that the Home Office are preparing a test case relating to the use of the technique to be brought before the English criminal courts. It is important, therefore, that lawyers acquaint themselves with the general principles involved in the technique and its implications for both criminal and civil law.

The technique[2]

Human cells contain within them all of the information needed to produce a complete human body. This human blueprint is carried in discrete packets

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[1] See B. E. Dodd, "DNA fingerprinting in matters of family and crime" (1985) 318 *Nature* 506.

[2] By necessity the treatment of technical information has been simplified for the lay reader. Those wishing a more comprehensive treatment should consult D. J. Weatherall, *The New Genetics and Clinical Practice* (2nd ed., 1985).

of information known as chromosomes, and the material of which they are made is called DNA. There are 46 such packets within a cell and they can be arranged by means of common characteristics (such as their appearance under the microscope) into 23 pairs. At fertilisation the ovum and sperm, each of which contain 23 single packets combine to produce the total of 46. Thus 50 per cent. of the genetic information is of maternal origin, and 50 per cent. is of paternal origin.

If one thinks of how information may be stored, many examples can be given, such as printed words on paper, grooves on a record or images on a photographic film. In all of these examples the information is stored on an inert medium but in the case of DNA the information is contained within its own chemical structure. Four building blocks known as bases make up DNA, and the order in which these bases occur provides the information required to assemble and regulate the construction of the body.

The bases are known by their initial letters, A, G, C, T and the structure of DNA itself can be thought of as resembling a zip fastener where A, G, C, T are the teeth of the fastener. Zips have two strands and so too has DNA, but unlike a zip, DNA will only close when A pairs with T, and G with C (as shown in fig. 1).

FIGURE 1

—A G C G G C A T T T A G G C A T A A—
—T C G C C G T A A A T C C G T A T T—

Fragment of DNA showing double strand structure and complementary base pairing.

Two other properties of DNA are important to our understanding of DNA fingerprinting.

1. The number of bases in a chromosome is enormous. It has been estimated that only about 45 per cent. of these are required for normal cell operation; the purpose of the remaining 55 per cent. is not yet understood. The functional units (the operative 45 per cent. of the bases) are spread throughout the length of the chromosome rather like pages of English text in an instruction manual. Between these pages of text are interspersed pages in an unknown language; we cannot understand them but they may be of great significance. If we were to represent a chromosome in the form shown in figure 1 it would require on average 1.3×10^8 characters to denote the entire length of the chromosome, that is at 5 characters per centimetre we would need a paper strip 261 kilometres long to write the entire base sequence. At intervals throughout the length, bases randomly occur in certain combinations of 6, known as restriction sites (see fig. 2).

[8] *The Times*, January 2, 1987.

FIGURE 2

—AAGCTT—
—TTCGAA—

Example of palindrome sequence of DNA recognised by restriction enzyme. (Many such palindromic sequences exist throughout the chromosome).

The sites are palindromic, *i.e.* the order of bases in the bottom strand is exactly the reverse of those in the top strand. Such sites occur throughout the DNA of the chromosomes, and, as characteristics of the DNA are inherited from the parents. Natural defence systems known as restriction enzymes occur in certain bacteria. These enzymes are capable of specifically recognising the palindromic sequences and breaking the DNA at that point. Restriction enzymes can be purified and used to break human DNA into fragments of different sizes which are separated from each other by a technique known as gel electrophoresis. The principles of this technique are briefly as follows:

DNA is placed in a small slot in one end of a gel, which is made in a mould (not unlike a fruit jelly) about 10 x 14 centimetres in size and about 0.5 centimetres thick. An electrical current is applied across the gel which causes the DNA fragments to move through the gel, and the distance moved by each fragment depends on its size. A picture can then be obtained where the fragments of DNA are seen ordered according to their size. The fragments can be transferred from the gel to a nylon paper-like material for further analysis. This procedure is known as "Southern blotting" after its inventor, Professor Ed Southern, and as the name suggests the fragments pass from the gel to the nylon sheet in a way similar to the movement of ink into blotting paper.

2. As well as containing restriction sites, the chromosome also has regions of repeated sequence throughout its length, that is, combinations of bases which occur again and again. A rough analogy might be the repetition throughout a telephone directory of a page of surnames or the recurrence throughout an instruction book of one particular page. In the case of the directory or instruction manual this would clearly be some kind of manufacturing error but no one knows why the sequences are repeated in the chromosomes. The important point here is that they exist and can be used to identify DNA fragments. How is this done? Earlier we said that DNA existed in two strands like a zip but that the teeth had to be precisely matched. Thus a strand of DNA:

STRAND (A) A G C C T G C T C T T A

could only match with its partner:

STRAND (B) T C G G A C G A G A A T

Separate complementary strands which match in this manner have an affinity for each other and will under suitable conditions bond ("hybridise") with each other to reform a double strand. This affinity is such that only minute amounts are required for hybridisation to occur, and this fundamental property of DNA is the key to the use of DNA

probes. In the above example sequence (B) will hybridise with sequence (A).

When the DNA is broken by a restriction enzyme and separated into fragments, many of these will contain a portion of repeated sequences since these regions occur throughout the chromosome. It is possible to purify these repeated sequences, and then label them with radioactivity to enable subsequent detection. These radioactive sequences are known as "probes" and hybridise with the repeat sequence present in the fragments separated on the gel. Thus if we were to expose sequence (A), blotted on a thin nylon membrane to a complementary sequence (B) (our "probe") the complementary strands of the zip will find each other and the radioactive strand will hybridise with its partner. When the nylon membrane is placed in contact with an X-ray film, the radioactivity will cause an image of bands to be produced on the film, remarkably like the bar code on the side of a food package.

Certain points remain to be made. As we mentioned earlier, chromsomes are derived from both parents so the distribution of restriction sites and hence the fragment separations are similarly inherited. In the process of egg and sperm formation some rearrangement of the DNA occurs, but at the same time large sections are left unaltered..Thus a son or daughter has DNA characteristics of both parents, but is a unique individual. When DNA is analysed by means of the repeat sequence probe, fragments can be detected which are of maternal and paternal origin, while others are specific to the child. DNA fingerprinting produces a band pattern as unique in its way as a normal fingerprint but from which in addition information relating to the parental origin can be derived.

Another use of DNA probes is in the prenatal diagnosis of genetic disease where a statistic is calculated from which the probability of a genetic disorder being inherited is derived. In contrast to this, in paternity disputes and criminal investigations, the technique will be used to make a positive identification. While many individuals share, for example, a particular blood group, the extracted DNA from a biological specimen will match exactly with the donor's DNA and nobody else's. This is confirmed by the DNA banding patterns which will be exactly the same for donor DNA and specimen DNA Thus a rape suspect could be positively and exclusively linked with a semen deposit. The only possible confusion lies in the case of identical twins who will share many DNA characteristics. The DNA banding patterns of ordinary siblings will show considerable differences.[3]

Applications

In 1983 a boy was refused entry into the United Kingdom because immigration officers doubted that he was the son of a Ghanaian woman who

[3] Detailed scientific discussion of DNA fingerprinting can be found in the following research articles: A. J. Jeffreys, V. Wilson. and S. L. Thein (1985) 314 *Nature* 67; A. J. Jeffreys, V. Wilson and S L. Thein (1985) 316 *Nature* 76; A. J. Jeffreys, J. F. Y. Brookfield and R. Semenoff (1985) 317 *Nature* 818; P. Gill, A. J. Jeffreys and D. J. Werrett (1985) 318 *Nature* 577. The successful application of the technique will require highly qualified and skilled laboratory staff at least in the initial stages of development.

held rights of settlement in this country. An appeal to an immigration adjudicator was unsuccessful on the grounds that the medical evidence, based on conventional tests, did not rule out his being the nephew of the woman in question rather than her son. In preparation for a further hearing before the Immigration Appeal Tribunal, the new DNA technique was employed. Statistical analysis of the DNA fingerprints established with virtual certainty that the woman was the boy's mother. This conclusion was based on the gel analysis which showed a high number of shared bands between the woman and boy. The chance of this match occurring at random is about 30,000 million to one, *i.e.* 30,000 million people would require to be tested before such a match would occur by chance (the world population at present is about 4,000 million!). The likelihood of an aunt and nephew sharing the same band patterns was half a million to one; certainly higher, but still low enough for the relationship to be excluded.

In spite of these remarkable figures the Home Office did not concede the case at this point. Apparently they doubted the ability of the technique to determine the mother–son relationship with such certainty. On the day of the hearing the case was conceded by the Home Office without the technique being legally tested or considered.[4] Nevertheless, the Home Office now appears to have changed their view of the technique. In a House of Commons written reply of October 28, 1986 the Home Office Minister, Mr. David Waddington, announced that the Government was planning to hold a trial applying DNA testing to a sample of about 40 immigration cases where a parent-child relationship is fundamental to the claim to enter or remain in the United Kingdom. The analysis is to be carried out by Dr. Jeffreys of the University of Leicester who was the developer of the technique. At the same time it was announced that preliminary discussions had been held with Imperial Chemical Industries about the commercial development of DNA testing.[5]

As stated earlier, it is reported that the Home Office may be preparing a test case to be brought before the criminal courts.[6] The result of such a case would be significant for a wide variety of legal issues. Already it has been reported that in November 1986 a youth who had been held in prison for three months on a charge of murder was freed by Leicester magistrates after use of the technique. The youth had been accused of sexually assaulting and strangling a girl aged 15. The magistrates were told that DNA tests showed that the accused was not the murderer.[7] Police investigating this and an earlier case have now asked about 2,000 men aged between 16 and 34 living in the area to provide blood and saliva samples in an attempt to trace the killer.[8]

The potential applications of the DNA fingerprinting techniques are varied and many. As regards the law, its use in the investigation of crimes such as

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[4] The authors are indebted to Sheona York of the Hammersmith and Fulham Community Law Centre for providing an account of this case.

[5] H.C. Deb., October 28, 1986, cols. 90–91.

[6] I. S. Lomax, "DNA Fingerprints—A Revolution in Forensic Science" (1986) L.S.Gaz. 1213.

[7] *The Times*, November 22, 1986.

[8] *The Times*, January 2, 1987.

assault or rape, and in the determination of paternity can be seen from the above cases. By using this technique, a DNA fingerprint can be constructed which is unique to a particular individual. Thus in rape cases, minute blood or semen specimens, even of considerable age, may yield the necessary DNA information to allow positive identification of the person from whom they have come. This may even be possible where the specimens are mixed, *e.g.* where vaginal secretions are mixed with semen. In the determination of paternity or maternity the case described above illustrates the certainty with which the technique can be employed.

The distinguishing feature of DNA fingerprinting compared with traditional tests is that positive indentification is virtually certain. Existing alternative tests generally serve only to exclude persons from the matter under investigation.

As indicated earlier, an alternative use of the technique lies in the prenatal diagnosis of certain inheritable genetic illnesses.[9] In trial studies, it has been determined with a high degree of certainty whether a foetus is a carrier of cystic fibrosis provided one affected child has already been born. It is similarly possible to diagnose certain muscular dystrophies and some blood disorders. In fact DNA probes may soon be introduced to diagnose certain thalassaemias on a regular basis, and in the future Huntington's chorea may be identifiable. This raises questions for the affected families, and in particular the question of abortion and its availability under the current law. A carrier of Huntington's chorea for example can normally expect 30 to 40 years of healthy existence before the onset of the symptoms and then ten to twenty years of progressive mental and physical debilitation and degeneration leading to death. One unpleasant but revealing statistic associated with Huntington's sufferers is a suicide rate up to fourteen times higher than for the general population (U.S. figures).[10] Under these circumstances, given a reliable prenatal diagnostic test, would it be right or lawful to abort? The technique will bring considerable advantages to forensic investigation, but its use as a diagnostic tool raises important moral and legal questions.

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[9] The technique used to prepare and separate the DNA is identical to that described earlier but the probes used are of a different nature.

[10] S. J. Bird, "Pre-Symptomatic testing for Huntingdon's Disease" (1985) 253 *Journal of the American Medical Association* 3286.

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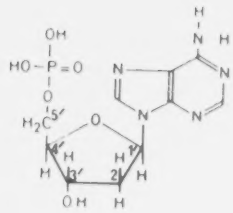
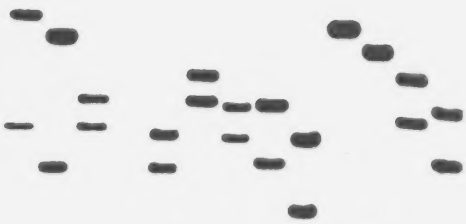
Federal Bureau of Investigation

Crime Laboratory Digest

Review Article In This Issue

Methods of DNA Analysis Under Investigation in the FBI Laboratory





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PUBLICATION POLICY

The Crime Laboratory Digest is published quarterly by the FBI Laboratory in cooperation with the American Society of Crime Laboratory Directors (ASCLD). It is inteded to serve as a rapid means of communication between crime laboratories, permitting information of interest and value to be disseminated among crime laboratory scientists.

Inclusion of an article in the Crime Laboratory Digest in no way represents an endorsement or recommendation of any part of that article by the Federal Government, the Department of Justice or the FBI. Contributing authors assume total responsibility for the contents and accuracy of their submission. Questions or requests concerning an article should be directed to the contributing agency.

All submissions are subject to editorial review in accordance with the editorial policy established by the FBI Laboratory and ASCLD. The editorial staff of the Crime Laboratory Digest reserves the right to edit all articles for style, grammer and punctuation. Comments and letters to the editor are encouraged and will be published when appropriate and as space permits. These should be forwarded to:

Crime Laboratory Digest - Editor
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FEATURE ARTICLE: INACTIVATION OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) BY GAMMA AND X-RAY IRRADIATION IN BODY FLUIDS AND FORENSIC EVIDENCE — PAGE 22

Paul D. Bigbee discusses the results of a collaborative research study by the FBI Laboratory, the National Cancer Institute and the National Bureau of Standards on the inactivation of the AIDS virus in body fluids and forensic evidence by gamma and x-ray irradiation. Also discussed is the revised policy of the FBI Laboratory regarding the submission of evidence known to contain or suspected of containing the AIDS virus.

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ON THE COVER

The cover, prepared by the Special Projects Section of the FBI Laboratory, depicts the structure of a DNA nucleotide, an autoradiogram of restriction fragment length patterns achieved by using a single locus DNA probe and a DNA double stranded helix.

CRIME LABORATORY DIGEST Vol 15, No. 1, January 1988
ISSN NO. 0743-1872

Message from the Assistant Director in Charge of the FBI Laboratory

The forensic analysis of DNA offers the promise of a positive personal identification of an individual based on a drop of blood or a single hair. Because of its ability to individualize, it has been referred to as "DNA fingerprinting." It represents perhaps the most significant forensic breakthrough of the century in its ability to identify a rapist or murderer, based on biological evidence left at the scene of the crime.

The FBI has initiated an aggressive forensic research program to develop this technology for eventual implementation in the FBI Laboratory. An ambitious technical training program is also being developed to impart knowledge of this technology for use by state and local crime laboratories throughout the United States. The FBI research effort is directed not only at methods development, but also at establishing the scientific validity and reliability of these methods to insure that evidence derived from the forensic analysis of DNA can withstand legal challenges to its introduction in the courts.

As you are aware, there are currently two private testing laboratories in the United States that are advertising the availability of DNA testing through their facilities. For your information, the FBI has adopted a policy that it will not submit evidentiary material for DNA testing at this time. This policy is in effect because we have not seen the results of systematic studies which assess the reliability of the methods used by private laboratories when the specimens are dried body fluid stains. The FBI Laboratory will not incorporate DNA typing procedures into casework examinations until the influences on DNA typing patterns are satisfactorily understood with respect to the environment, the presence of contaminants, aging effects and the mixing of body fluids.

DNA technology offers many intriguing potential applications for law enforcement. It has been proposed that DNA identification profiles of convicted sex offenders be collected and retained in an automated data system. As an aid in the investigation of unknown subject homicide and rape cases where the perpetrator's blood, semen or hair is found at the scene, DNA profiles could serve to identify and link serial offenses.

There are a number of policy considerations that must be addressed and strategies that must be developed within the FBI and the entire law enforcement community in order to establish this technology for use in the Nation's criminal justice system. Some states have already passed legislation which permits the collection of blood samples from convicted sex offenders. It is apparent that there is a critical need for a well coordinated effort in order to establish a viable, cooperative DNA data bank. The FBI is developing proposals to present to the crime laboratory and law enforcement communities intended to facilitate the rapid and ready exchange of DNA technical data. You are invited to present your views and concerns on the implementation of this dramatic and important emerging technology.

ROGER T. CASTONGUAY

Editor's Column

The review article in this issue, "An Introduction to the Methods of DNA Analysis Under Investigation in the FBI Laboratory," by Drs. Budowle, Deadman, Murch and Baechtel, serves the twofold purpose of providing an overview of the rapidly developing DNA technology as well as providing information on the DNA research effort of the FBI Laboratory. As this research effort progresses, we will keep the forensic community advised through this publication as to the new research developments and eventual training and on-line implementation of DNA analysis by the FBI Laboratory.

Also in this issue is the third article to appear in the Crime Laboratory Digest on acquired immunodeficiency syndrome (AIDS) and the forensic laboratory. This article by Paul Bigbee discusses the results of collaborative research between the FBI Laboratory, the National Cancer Institute and the National Bureau of Standards on the inactivation of the AIDS virus in body fluids and forensic evidence by gamma and x-ray irradiation. Also discussed is the policy of the FBI Laboratory pertaining to submission of evidence known to contain or suspected of containing the AIDS virus.

For those individuals interested in submitting information for inclusion in the Crime Laboratory Digest, we are now able to accept this information via telecommunications. Please telephone the editor on 703-640-1126 to make the necessary arrangements.

CRIME LABORATORY DIGEST SUBSCRIBER MAILING LIST

We are updating our current mailing list for the Crime Laboratory Digest and request that you complete the form on page 29 and return it to us in order that we can keep you on the mailing list.

ASCLD MEMBERSHIP

Mr. Edward E. Hueske, Chairman of the Membership Committee of the American Society of Crime Laboratory Directors (ASCLD), is seeking new members for ASCLD. Applications for membership can be obtained by contacting Mr. Edward E. Hueske, Chairman, ASCLD Membership Committee, Arizona Northern Regional Crime Laboratory, P.O. Box 15500, Flagstaff, Arizona 86011 (telephone: 602-779-5176).

TO ASCLD MEMBERSHIP

Per the charge to the ASCLD Publications Committee, it is required that the ASCLD membership be queried periodically for comments and constructive criticism relative to the editorial policy of the Crime Laboratory Digest. Responses to the following questions, as well as additional comments will be appreciated and should be sent to David Stafford, Chairman, ASCLD Publications Committee, Toxicology Laboratory, University of Tennessee, Memphis, TN 38613.

In responding, please keep in mind that the primary thrust of the Crime Laboratory Digest is to disseminate information to the bench level examiners. A copy of the February 12, 1988, committee report was submitted to the ASCLD secretary for inclusion in the minutes of the February ASCLD board meeting. Please address the following questions and comments:

1. Are you receiving the Crime Laboratory Digest and making it available to your personnel?

2. Is the Crime Laboratory Digest helping to meet the needs of your examiners? If not, what suggestions do you have to improve the situation?

3. Have you encouraged your personnel to advise the Publications Committee of the subject areas or issues they would like to see addressed as review or technical articles and perhaps who they believe might be appropriate to write such articles? If not, please do so!

4. Have you encouraged your personnel to submit articles on current projects which might be useful to other examiners? This would include any research efforts or information of potential benefit to others.

5. The Crime Laboratory Digest is a refereed publication, and all copy submitted is equitably reviewed. Not all material submitted is published; the editors, with recommendations from reviewers, determine which submissions are acceptable for publication. The policy has always been to help submitters where clarification is necessary.

6. Do you have any comments or constructive criticism not covered in 1-5? Specific examples are most helpful in addressing weaknesses and making improvements.

Your early response and participation will be appreciated. Current members of the ASCLD Publications Committee include:
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John Murdock, Martinez, California
Gerald Smith, Knoxville, Tennessee
David Stafford, Memphis, Tennessee

EMPLOYMENT OPPORTUNITIES

Chief Criminalist - Physical Evidence, Firearms Identification: The city of Charlotte is accepting applications for two Chief Criminalist positions, one in Physical Evidence and the other in Firearms Identification. Qualifications for the Chief Criminalist in Physical Evidence position include a Bachelor's degree, with major course work in chemistry, biology or criminalistics and experience in chemical/analytical laboratory work with a law enforcement agency. Duties include recognition, identification, analysis and evaluation of numerous types of trace physical evidence, rendering court testimony and supervision of crime laboratory personnel.

Qualifications for the Chief Criminalist in Firearms Identification position include a Bachelor's degree, with experience in toolmark and firearm identification or equivalent laboratory experience. Duties include examination, repair and identification of firearms, report writing, rendering court testimony and supervision of crime laboratory personnel. Salary range for both positions: $29,226 - $41,124 per year. Submit applications to the Personnel Department, City of Charlotte, 600 East Trade Street, Charlotte, North Carolina 28222 (telephone: 704-336-2287). The city of Charlotte is an Equal Opportunity Employer.

Criminalist II: The city of Charlotte is accepting applications for a court qualified Criminalist II to operate a one-person Questioned Documents Unit. Qualifications include a Bachelor's degree in a natural science and excellent communication skills. Candidates must be qualified (or attainment of same within 2 years) by the American Board of Questioned Documents and have prior court qualification as an expert witness in document examination. Duties include examination and rendering written opinions and court testimony in all aspects of questioned documents examinations. Salary range: $26,509 - $37,300 per year. Submit applications to the Personnel Department, City of Charlotte, 600 East Trade Street, Charlotte, North Carolina 28202 (telephone: 704-336-2287). The city of Charlotte is an Equal Opportunity Employer.

Criminalist III: The city of Mesa is currently seeking applicants for the position of Criminalist III. Qualifications include any combination of training, education or experience equivalent to a Bachelor's degree in chemistry, criminalistics or a closely related physical or natural science and 6 years of experience as a working Criminalist. Experience in courtroom expert testimony is required. Duties include examination, identification and evaluation of physical evidence by utilizing a range of chemical, microscopic and chromatographic techniques. Salary range: $33,514 - $45,214 per year (1-year grant-funded position with the possibility of it becoming full-time). Submit applications to the City of Mesa Personnel Department, 64 East First Street, P. O. Box 1466, Mesa, Arizona 85211-1466 (telephone: 602-834-2367). The city of Mesa is an Equal Opportunity Employer.

Criminalist III: The Law Enforcement Division of the State of South Carolina is seeking applicants for the position of Criminalist III as a Firearms-Toolmark Examiner. Minimum qualifications are a Bachelor's degree plus 3 years of full-time experience as a firearms-toolmarks examiner in a recognized forensic laboratory or a Master's degree plus 2 years of full-time work experience. Duties include performing physical and microscopic analysis of firearms-toolmark physical evidence, report writing and presenting court testimony. Salary range: $25,365 - $30,846 per year. Candidates should submit applications to Lieutenant Dan DeFreese, South Carolina Law Enforcement Division, P.O. Box 21398, Columbia, South Carolina 29221 (telephone: 803-737-9040). The State of South Carolina is an Equal Opportunity Employer.

Document Examiner: The city of Chicago is currently seeking applicants for the position of Chief Document Examiner. Minimum qualifications include a Bachelor's degree in a physical science or related course of study, supplemented by 3 years of work experience in document examination which should include 1 year in supervision or an equivalent of training and experience. Position duties include examining the full range of evidence classified as questioned documents. Salary range: $29,280 - $40,644 per year. Submit applications to Sergeant Frank Kehoe, Crime Laboratory Division, 1121 South State Street, Chicago, Illinois 60605 (telephone: 312-744-5526). The city of Chicago is an Equal Opportunity Employer.

Evidence Technician: The city of San Diego is recruiting candidates for an Evidence Technician position. Minimum qualifications consist of any of following: 1) a combination of full or part-time experience which equals 1 year of full-time experience as an evidence technician and must include experience in investigative photography; 2) a combination of full or part-time experience which equals 2 years of full-time experience in investigative photography (surveillance, shoe and tire impression, document, fingerprint or crime scene photography); or 3) completion of an Associate of Science degree or a Community College certificate in Evidence Technology. Applicants with color darkroom experience are preferred. Duties include the full range of duties normally associated with the position of evidence technician, including presenting courtroom testimony. Salary range: $1,957 - $2,364 per month. Candidates should contact Fred Gallardo, Personnel Department, City of San Diego, 202 C Street, San Diego, California 92101 (telephone: 619-236-5753). The city of San Diego is an Equal Opportunity Employer.

Forensic Chemist: The Denver Police Department is currently seeking applicants for a court qualified Forensic Chemist. The position requires a Bachelor's degree in chemistry, biochemistry or forensic chemistry, supplemented by 2 years of forensic laboratory experience. Position duties include the collection and examination of physical evidence using modern, sophisticated laboratory instrumentation, report writing and expert witness testimony. Salary range: $2,537 - $3,171 per month. Submit applications to Don Braden, Career Service Authority, 414 14th Street, Denver, Colorado 80202. The Denver Police Department is an Equal Opportunity Employer.

Serologist or Hair Examiner: The Fort Worth Police Department Crime Laboratory is seeking candidates for a Serologist or Hair Examiner position. Qualifications include a Bachelor's degree in a biological, chemical or related science plus 2 years of specialized laboratory experience. Position duties include the examination of body fluids and stains using standard serological procedures for blood grouping and enzyme variant determinations. Alternately, the position duties will be tailored to include hair examinations and serological testing, depending upon qualifications of the examiner. Salary range: $24,780 - $34,344 per year. Submit applications to Frank Shiller, Director, Fort Worth Police Department Crime Laboratory, 330 West Belknap Street, Fort Worth, Texas 76102 (telephone: 817-877-8084). The city of Fort Worth is an Equal Opportunity Employer.

Supervisor, Forensic Sciences (Serology): The Rhode Island Department of Health is seeking qualified candidates for a Supervisor of Forensic Sciences (Serology). Minimum requirements include a Master's degree in the field of specialty and related supervisory experience in a forensic laboratory. Substantially equivalent qualifications will be considered. This position is subject to the provisions of the Rhode Island Merit System Law and is subject to civil service examination. Duties include supervision and operation of a forensic serology laboratory, blood grouping, species/sex determination, serological examinations and presentation of expert witness testimony. Salary range: $31,906 - $36,005 per year. Submit applications to Dr. David B. Uliss, Chief, Toxicologist, Rhode Island Department of Health, Division of Laboratories, Chapin Laboratory Building, 50 Orms Street, Providence, Rhode Island 02904 (telephone: 401-274-1011). The State of Rhode Island is an Equal Opportunity Employer.

PROCEEDINGS OF INTERNATIONAL SYMPOSIA PUBLISHED BY THE FBI LABORATORY

The following proceedings of international symposia hosted by the FBI Laboratory are currently available from the Government Printing Office:

The Proceedings of a Forensic Science Symposium on the Analysis of Sexual Assault Evidence, stock number 027-001-00037-8, price $9.50 (United States), $11.90 (foreign delivery).

The Proceedings of the International Symposium on the Analysis and Identification of Polymers, stock number 027-000-01265-5, price $9.50 (United States), $10.50 (foreign delivery).

The Proceedings of the International Symposium on Forensic Hair Comparisons, stock number 027-001-00044-1, price $11.00 (United States), $13.75 (foreign delivery).

The Proceedings of the International Symposium on Questioned Documents, stock number 027-001-00043-2, price $9.50 (United States), $11.90 (foreign delivery).

To obtain copies of these publications, inquiries should be directed to:

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SPECIALIZED SCIENTIFIC SCHOOLS FY 88

The October 1987 issue of the Crime Laboratory Digest listed the specialized scientific courses being offered by the FBI in Fiscal Year 1988. Two dates have changed and the new information is as follows:

( ) Indicates Number of Students Per Class

Police Arson Crime Scene Investigators (24)
4/3 - 8/88

Chromatographic Methods in Forensic Science (12)
6/12 - 24/88

FORENSIC SCIENCE DATABASE

The Forensic Science Database (FORS), produced by the Central Research Establishment, Home Office Forensic Science Service, Great Britain, is now available commercially through the database vendor, Data-Star.

The Forensic Science Database indexes literature relevant to the examination of evidential materials, analytical methods and the presentation and interpretation of findings. The database coverage includes, but is not limited to, biology, chemistry, drugs, pathology, toxicology, documents and firearms examination as well as case law. Over 1,800 international sources including journals, books, conference proceedings, technical reports and government publications are indexed in the database. The database spans from 1976 to the present and is updated monthly. The Forensic Science Database is a valuable resource not only to forensic scientists but also to other professionals involved in criminal investigation and the provision of expert testimony to the courts.

To obtain additional information about the database, contact Data-Star or Colleen Wade, FBI Laboratory Division Librarian.

Data-Star
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Wayne, PA 19087
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FOOTWEAR AND TIRE IMPRESSION BIBLIOGRAPHY

A bibliography on footwear and tire impressions has been compiled by Colleen Wade, Librarian, and Special Agent William J. Bodziak, FBI Laboratory Division. Permission has been granted by the publishers to disseminate copies of the documents cited in the bibliography. This service is provided for all duly authorized crime laboratories. To obtain copies of the documents, send a written request to the following address. Supplies are limited. All requests must be received by July 15, 1988.

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CENTRAL RESEARCH ESTABLISHMENT REPORTS

The Central Research Establishment (CRE), Home Office Forensic Science Service, Great Britain, provides the FBI Laboratory with copies of the CRE Reports on completed research projects. These reports are available to all duly authorized crime laboratories in the United States. To obtain copies of the reports, send a written request to the following address. Supplies are limited. All requests must be received by July 15, 1988.

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The following CRE Reports are now available:

The Persistence on Footwear of Some Common Fire Accelerants, H. M. Carrington.
CRE Report #614

A New Method for Sex Determination of the Donor of Forensic Samples Using a Recombinant DNA Probe, P. Gill.
CRE Report #615

Colour Matching Within a Fibre Data Collection Using Complementary Chromaticity Coordinates, A. W. Hartshorne and D. K. Laing.
CRE Report #616

Comparison of Packed, Wide Bore Capillary and Narrow Bore Capillary Columns for the Screening of Drugs and Poisons in Forensic Samples, M. Japp, R. Gill, M. D. Osselton and J. Cordonnier.
CRE Report #617

An Evaluation of Three Prototype Handling Devices for Small Samples, I. Kirkwood and M. D. J. Isaacs.
CRE Report #618

Introducing the Bayesian Approach to the Evaluation of Scientific Evidence. Part 1, I. W. Evett.
CRE Report #619

A Comparison of the Isoelectric Focusing Patterns of Red Cell Phosphoglucomutase (PGM1) Separated on an Ampholine, Ampholine/Separator and Immobilised pH Gradient, R. M. Burgess, J. G. Sutton and D. J. Werrett.
CRE Report #620

Introducing the Bayesian Approach to the Evaluation of Scientific Evidence. Part 2, I. W. Evett.
CRE Report #621

Statistics Bibliography, S. R. Baker.
CRE Report #622

ELDAK. Data Acquisition and Analysis Software Suite for ELISA Research and Development, S. M. Fletcher.
CRE Report #623

Analysis of Alpha-2-HS-glycoprotein (A2HS) in Plasma and Bloodstains. Part II. Neuraminidase Treatment of Samples and the Discovery of a New Allele, A2HS*11, Found in Afro-Caribbeans, S. A. Westwood, P. J. Seaman, L. J. Thorogood, P. J. Ablett, I. Yuasa, S. Weidinger and K. Umetsu.
CRE Report #624

The Analysis of Alpha-2-HS Glycoprotein in Plasma and Bloodstains by Isoelectric Focusing. Part III. The Phenotypic Frequencies of Alpha-2-HS-glycoprotein and Group Specific Component in 3 Ethnic Groups. The Use of These Proteins as Racial Markers in Forensic Biology, S. A. Westwood, P. J. Seaman, C. O'Brien and L. J. Thorogood.
CRE Report #625

Introducing the Bayesian Approach to the Evaluation of Scientific Evidence. Part 3, I. W. Evett.
CRE Report #626

Background Volatiles in Fire Debris I. Burnt Carpet - Preliminary Results, P. A. Rapley.
CRE Report #627

On the Ignition of Petrol by Smouldering Tobacco, J. A. Zoro, R. H. Ide and M. A. Payne.
CRE Report #628

The Characterisation of Acid Dyes in Forensic Fibre Analysis by HPLC Using Narrow-bore columns and Diode Array Detection, D. K. Laing, R. Gill, C. Blacklaws and H. M. Bickley.
CRE Report #629

The Examination of Paint and Fibres as Thin Sections, J. M. Wilkinson, R. A. Rickard, J. Locke and D. K. Laing.
CRE Report #631

A Data Collection of Vehicle Topcoat Colours Made with the Johne and Reilhofer ER10 Colorimeter, J. Locke, J. M. Wilkinson and T. J. Hanford.
CRE Report #632

Simplified ABH Grouping of Bloodstains by an Auto-Analyser Technique, E. R. Rutter.
CRE Report #634

Evaluation of the Non-specific Displacement Effects of Various Detergents, Food Substances, Dyes and Ex-case Work Semen Free Fabrics on the RIA of 190H F1alpha/F2alpha PG, J. G. Sutton, L. Trewsdale and S. J. King.
CRE Report #635

The Effect of Neuraminidase Treatment on the Detection of Lewis a and Other Lewis Related Factors in Semen Stains by ELISA, B. G. Lang.
CRE Report #636

Sample Handling and Storage for the Quantitative Analysis of Toluene in Blood by Headspace Gas Chromatography, R. Gill, S. E. Hatchett, M. D. Osselton, H. K. Wilson and J. D. Ramsey.
CRE Report #637

Review Article

An Introduction to the Methods of DNA Analysis Under Investigation in the FBI Laboratory

Bruce Budowle
Harold A. Deadman
Randall S. Murch
F. Samuel Baechtel

Forensic Science Research and Training Center
Laboratory Division
FBI Academy
Quantico, Virginia 22135

For more than 2 decades, the ability to resolve and detect polymorphic markers has made possible the genetic characterization of body fluid stains by forensic scientists. Although the polymorphic protein and antigen markers employed by many laboratories provide the potential for a high degree of discrimination among different individuals, this upper limit is attained infrequently because of the instability of some of these markers in dried stains. Moreover, of the markers that retain their structure and activity in the dried state, the number of expressed or observed forms is limited. Thus, in practice, the individualization of many evidentiary stains cannot be carried out to any great extent given the present array of polymorphic markers .

Advances in recombinant DNA technology have provided the molecular tools that enable scientists to detect the extraordinary variability that exists among individuals at the level of their deoxyribonucleic acid (DNA). This technology holds promise of affording the forensic serologist the ultimate in discrimination power, the ability to identify a body fluid/tissue donor to the exclusion of all other individuals.

The goal of this document is to introduce crime laboratory personnel to the principles of DNA analysis that enable the detection of polymorphisms at the level of the genetic code. The methodology featured is that being actively investigated by the FBI Laboratory. In doing so, we trust that the reader will become more familiar with the vocabulary of molecular biology and appreciate the power of this approach for the genetic characterization of human biological specimens.

STRUCTURE OF DNA

DNA is a polymer composed of four building blocks called nucleotides. A nucleotide contains deoxyribose, inorganic phosphate and a nitrogenous base (Figure 1).

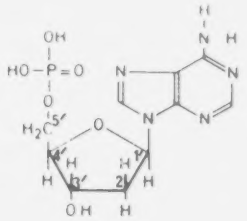

Figure 1. Structure of a nucleotide. Illustrated is deoxyadenosine-5'-monophosphate. Note the linkage of the adenosine to the 1' position and the phosphate to the 5' position of the deoxyribose.

Four different nucleotides are found in DNA, and these differ in the type of nitrogenous base that is covalently linked to deoxyribose. Purine nitrogenous bases are adenine (A) and guanine (G). The pyrimidine bases in DNA are cytosine (C) and thymine (T) (Figure 2). The nucleotides in DNA are bound together covalently via phosphoester linkages between the 5' carbon of deoxyribose of one nucleotide and the 3' carbon of the deoxyribose in the adjacent nucleotide (Figure 3). The four different bases can be attached in any order along the phosphate-deoxyribose backbone. An enormous array of different sequences of nucleotides can exist in a single strand of DNA that is hundreds of thousands of nucleotides long. For example, if we

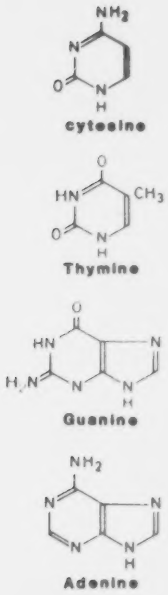

Figure 2. The four nitrogenous bases found in DNA. Cytosine and thymine are pyrimidines, and guanine and adenine are purines.

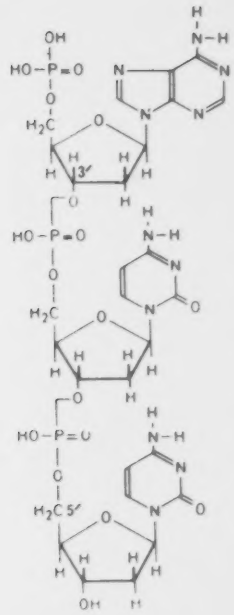

Figure 3. Linkage pattern of the nucleotides in a single strand of DNA. Note that the phosphate group is linked to the 3' and 5' positions of successive deoxyribose moieties.

consider only 10 positions in the chain, each of which could be occupied by any 1 of the 4 nucleotides, the number of combinations would be 4^{10} or 1,048,576.

The DNA that is located in the cell nucleus is composed of two polynucleotide chains that associate in a very particular manner to form a helical molecule (Figure 4). The association between the two strands results from the formation of hydrogen bonds between specific bases on the separate strands. The purine base A in one polynucleotide chain will only form hydrogen bonds with the pyrimidine base T in the other strand. Likewise, the G and C in opposite strands will associate by hydrogen bonding (Figure 5). The association of a single pyrimidine with a single purine through hydrogen bonding is referred to as a base pair (bp). For example, if the sequence on one strand were 5'-T-T-G-C-A-C-T-T-A-3', the sequence on the opposing strand would be 3'-A-A-C-G-T-G-A-A-T-5'. Opposing strands of DNA that hydrogen bond with one another are referred to as being complementary. Complementary base pairing of DNA strands contributes to the stability of the duplex molecule.

The phenomenon of complementary base pairing is exploited in all DNA typing methodologies. Exposure of DNA to specific temperatures or an alkaline environment will abolish the hydrogen bonding and cause the two strands to separate. The process of separating double stranded DNA into its component single strands is referred to as denaturation. Although the two strands can be separated in this fashion, the

Figure 4. The DNA double helix. Two single polynucleotide chains are held in association by hydrogen bonds to form the double stranded helix.

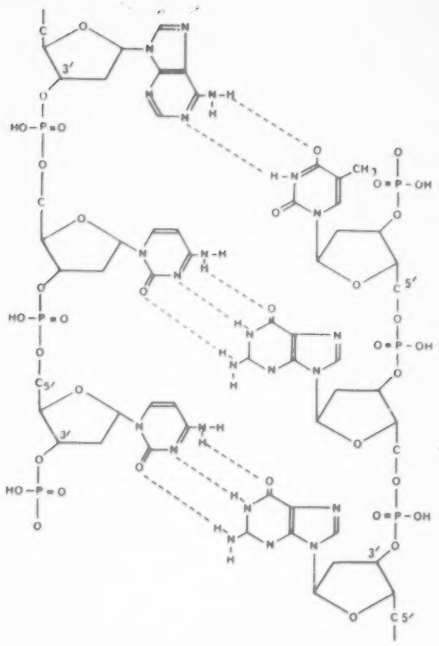

Figure 5. An illustration of the hydrogen bond patterns between complementary strands of DNA.

phosphoester bonds that link the nucleotides of each strand are unaffected. Under the appropriate conditions, two sections of single-stranded DNA that contain complementary sequences can be hybridized (annealed) to form double stranded DNA.

Most of the DNA of human cells is organized and packaged into the 46 chromosomes of the nucleus. Only a portion of the chromosomal DNA codes for the synthesis of proteins; the remaining DNA serves either in a regulatory capacity or does not serve any known coding or regulatory function.

RESTRICTION ENDONUCLEASES

Restriction endonucleases (RE)s are enzymes derived from bacteria that have the ability to catalyze the cleavage of double stranded DNA. REs fall into three general categories based on the manner by which they sever the DNA strands. Only class II REs are used in the DNA typing methods discussed in this document. Class II REs will cleave DNA only at specific recognition sites. A particular RE will cleave DNA only if a specific sequence of bases is present. This recognition sequence is referred to as

the restriction site for that RE. For example, the RE named Hinf I recognizes the nucleotide sequence GANTC (N implies that any of the four nucleotides can occupy this position in the sequence); RE Eco RI recognizes the sequence GAATTC.

A separate consideration is the manner by which the RE actually severs the DNA strands once recognition has been achieved. Some REs will sever directly across the DNA strands. RE Hpa I recognizes the sequence:

5'-G-T-T | A-A-C-3'
3'-C-A-A | T-T-G-5'

and cuts the DNA strands such that even, or blunt ends result on the fragments. In contrast, RE Eco RI cuts each of the DNA strands at offset positions in the recognition sequence:

5'-G | A-A-T-T-C-3'
3'-C-T-T-A-A | G-5'

When an RE such as Eco RI cuts DNA, the ends of the fragments are not blunt, but one or more nucleotides are left hanging unpaired. Cuts of this nature are referred to as cohesive or sticky ends. Cohesive ends facilitate the ligation of unrelated DNA fragments to form longer strands of this material.

DNA PROBES

A probe is a short single strand of DNA, labeled with a reporter molecule. When the probe is combined with single-stranded DNA, it will hybridize with that portion of the DNA that contains complementary nucleotide sequences.

The development of a DNA probe begins with the digestion of genomic DNA by a RE that will produce many DNA fragments. A RE is chosen that will yield cohesive ends on the fragments. Through the action of a DNA ligase, these fragments can be inserted into extrachromosomal bacterial DNA elements such as plasmids that have been linearized by the same RE. The insertion of human DNA fragments into bacterial plasmids forms a recombinant DNA molecule. Plasmids are separate circular pieces of intracellular bacterial DNA that are replicated autonomously from the bacterial genome itself. By insertion of a DNA sequence into a plasmid, the number of copies of this recombinant DNA molecule (and the inserted sequence as well) will be amplified into many individual copies as the bacteria grow during culture. After harvesting the bacterial DNA and isolation of the plasmids, the original sequence insert can be removed from the plasmids by reapplication of the appropriate RE. The net effect of this process, which is known as cloning, has been to increase enormously the number of separate copies of the particular sequence. These copies of the sequence, when tagged with some reporter molecule, are known as probes. Plasmids are one category of vectors that can be employed for the cloning of recombinant DNA molecules. Other categories of vectors are cosmids and bacteriophages (bacterial viruses). Vectors must possess certain characteristics to be effective as carriers of recombinant DNA. Vectors must carry genes that confer an easily recognized phenotype (i.e. antibiotic resistance) upon the bacterial host so that cells containing the vector can be preferentially selected out. A vector must possess a single RE cleavage site outside the essential replicating region where the foreign DNA can be inserted. Finally, a vector must be replicated autonomously within the host bacterium so that amplification of the specific DNA fragment can be achieved.

Because the probes are exact copies of a particular sequence of DNA, they will be able to engage in complementary base pairing with the original nucleotide sequences in genomic DNA. This procedure is the foundation upon which is based the detection of allelic differences in genomic DNA.

For use in detecting polymorphic regions in genomic DNA, probes can be tagged with a radioisotope. Radioisotope labeling of probes is effected when a copy of the sequence is made using DNA polymerase and the four nucleoside triphosphates. One of the nucleoside triphosphates (often dCTP) has the radioactive isotope of phosphorus (^{32}P) present at the alpha phosphate position in the molecule. As the polymerase copies the probe, the radioactive isotope is incorporated into the DNA molecule where it will identify the physical location of the probe.

DETECTION OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS

Restriction fragment length polymorphisms (RFLPs) are variations in the sizes of DNA fragments produced by RE digestion of genomic DNA. Although RFLPs differ in size, they contain similar or homologous nucleotide sequences. To generate and detect the RFLPs carried in the DNA of an individual, the DNA first must be isolated from an appropriate body fluid or tissue. Generally, this can be accomplished by digestion of the specimen with a proteolytic enzyme in the presence of a detergent, followed by one or more organic extractions to remove denatured protein. Once isolated and freed of protein, the DNA is suitable for cutting with REs. After digestion with REs, the resulting DNA fragments are fractionated on a size basis by electrophoresis through an agarose gel. Smaller DNA fragments will migrate faster than larger fragments because the gel matrix exerts a sieving effect on the DNA molecules during electrophoresis. The resolved DNA fragments in a gel containing ethidium bromide, which intercalates with DNA, will fluoresce under ultraviolet light. At the completion of electrophoresis, there are so many fragments per human genomic DNA specimen that the sample track appears as a smear (Figure 6). At this stage of the analysis, no fragments indicative of polymorphisms can be seen. Only through application of the DNA probe can the allele sequences be detected amidst the background of unrelated DNA fragments.

The array of DNA fragments in the gel is transferred to a nylon or nitrocellulose membrane by a process known as Southern blotting (Southern 1975). Southern blotting relies on the flow of a transfer solution from a reservoir through the agarose gel to a stack of dry paper towels or blot pads by capillary action. The membrane is placed between the gel and the paper towels. The DNA fragments are carried along with the flow of transfer solution out of the gel and onto the membrane where they bind readily. At some point, the DNA fragments must be denatured to yield single stranded DNA on the membrane. Some procedures effect denaturation of DNA prior to its transfer to the membrane, while other methods utilize alkali as the transfer solvent which denatures DNA during transfer.

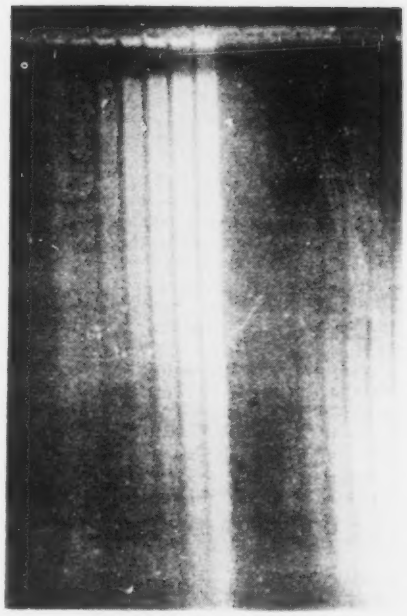

Figure 6. Agarose gel after electrophoresis of RE digested DNA. Ethidium bromide contained in the gel intercalates with DNA and fluoresces upon illumination with ultraviolet light.

Following an overnight washing in a prehybridization solution that blocks unoccupied binding sites on the membrane, the membrane is exposed to the radioactively-labeled probe. The probe will hybridize with the membrane-bound denatured DNA fragments that contain complementary nucleotide sequence. It should be stressed that not all hybrids between the probe and the target DNA fragments are perfect hybrids. Some of the hybrids will possess a degree of bp mismatches that result in less stable duplex molecules. Membrane washing procedures after probing control the degree of

mismatches in the hybrids. Washing the hybridized membranes under conditions of low stringency (high salt, low temperature) will leave a greater degree of mismatches within hybrids than washing at high stringency (low salt, elevated temperature) which removes probes that are weakly hybridized because of mismatches. The degree of mismatches acceptable in the hybrids will depend on the nature of the RFLPs being sought in the genomic DNA.

After the washing steps have been completed, the membrane is placed in intimate contact with a photographic film that is sensitive to the decay products of the radioactive phosphorus molecules. After an appropriate exposure time (several hours to several days), the film will bear a photographic image of the positions of the radioactive probe-target DNA hybrid array on the membrane. The resulting image can be evaluated either visually or by image analysis.

DNA POLYMORPHISMS

Point Mutations

Restriction sites are distributed randomly throughout the human genome. Digestion of DNA with a RE will result in the production of thousands of fragments of DNA that differ in length depending upon the distances between restriction sites.
The pattern, or array, of DNA fragments produced by a specified RE will be characteristic of (but not necessarily unique) for a given source of DNA, because the location of restriction sites can vary greatly among individuals. The variation in restriction site locations arises from mutational events (point mutations) that lead to the creation and/or elimination of these sites in the DNA of the germ cells.
A generic example of RFLPs is shown in Figure 7 in which the restriction sites are defined by the arrows. For allele A, there are 12,000 bps between the restriction sites, but for allele B a point mutational event has occurred so that there is an additional cleavage site. Since the probe hybridizes with only one end of the fragments, a smaller fragment will result upon RE digestion of allele B. A specific example of point mutation in humans is that which has occurred in the beta-globin gene and has led to the occurrence of sickle-cell anemia (Studencki *et al.* 1985). Within the nucleotide sequence that codes for the production of the normal hemoglobin molecule is the sequence 5'-C-C-T-G-A-G-G-A-G-3'. Individuals afflicted with sickle-cell disease carry the sequence 5'-C-C-T-G-T-G-G-A-G-3', in which only a single base change has taken place (a T has been substituted for an A). The RE Mst II recognizes and cleaves the DNA sequence 5'-C-C-T-N-A-G-G-3'. Thus Mst II can cleave the sequence in the normal gene, but cannot cleave the sequence found in the sickle-cell gene. Analysis of fragment lengths would reveal a 1150 base fragment for the normal hemoglobin sequence but a

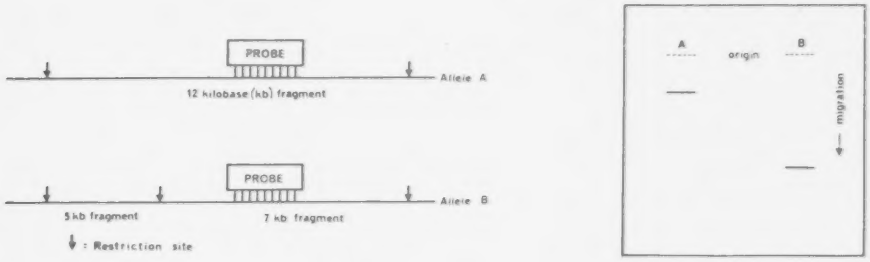

Figure 7. (Left) Generic example of a RFLP caused by a point mutation. The arrows indicate restriction sites. (Right) Location of the restriction fragments after gel electrophoresis.

1350 base fragment for the sickle-cell gene because the latter lacks one restriction site.

Although RE digestion is the most straightforward approach to screen for potential variation among individuals, it is not the most discriminating. Considerably more individual variation exists within the DNA sequences between restriction sites.

Repetitive Sequences

Observations by Wyman and White (1980) revealed that between restriction sites and within noncoding regions of genomic DNA were multiple repeats of certain nucleotide sequences. Moreover, the number of repeats of these core sequences was shown to be highly variable within the human population. For example, near to the DNA sequences that code for alpha-globin synthesis is a 17 bp sequence that can be present in the human genome in as few as 70 and as many as 450 repeats. Thus, the total number of bps in this DNA region would vary from 1190 (17 X 70) to 7650 (17 X 450). The variation in number of repeats of the core sequences may have occurred during unequal crossing over of chromosomes during meiosis. Because the number of repeats of these regions is inherited in a Mendelian fashion, a repeat sequence can be considered as an allele based on size. These size alleles are expressed codominantly. Some repeat sequence alleles are located solely at one locus on a pair of homologous chromosomes, whereas others are located at loci on multiple chromosomes.

Variable Number Tandem Repeats

Variable number tandem repeat (VNTR) sequences are those which are confined to a single locus on homologous chromosomes. Wyman and White (1980) described the polymorphism exhibited by this category of repeat sequences. Subsequently, similar polymorphisms have been observed in the introns associated with the insulin gene (Bell *et al.* 1982), Harvey ras oncogene (Capon *et al.* 1983) and myoglobin gene (Weller *et al.* 1984). Some VNTR sequences have been reported to exhibit a degree of polymorphism such that almost all of the population is heterozygous. When human genomic DNA RE fragments are examined with a VNTR probe, a maximum of two fragments will be detected on the membrane per individual. One fragment will have been derived from each of the VNTR loci on each of a pair of homologous chromosomes. VNTR probings are performed under conditions of high stringency so that a limited number of bp mismatches are tolerated.

The number of VNTR alleles at a particular locus is limited. Thus, fragment patterns derived from the application of a single VNTR probe do not enable the individualization of a DNA specimen. Rather, several VNTR probes, which recognize different VNTR loci, must be used to achieve individualization. VNTR probes have been applied successfully to the examination of body fluid stains by Giusti *et al.* (1986) and Kanter *et al.* (1986).

An example of the fragment pattern achieved with VNTR probes is illustrated in Figure 8. The two bands obtained from VNTR probing of DNA from a heterozygous individual will differ in their intensity on an autoradiogram. Because the faster moving DNA fragment possesses fewer copies of the repeat sequence, it will hybridize with fewer probe molecules (which recognize the core repeat sequence) and yield a less intense autoradiogram signal than will the

Figure 8. Autoradiogram of restriction fragment length patterns using a single locus DNA probe (YNH24).

larger fragment that contains more copies of the repeat sequence. The implication of this behavior of probes is that for a forensic sample that contains a limited quantity of DNA, the smaller fragment may not appear on the autoradiogram due to the small quantity of labeled probe that was bound. Such a specimen might be typed as having originated from an individual who is homozygous at a particular VNTR locus, when in fact he was heterozygous. Thus, it may be necessary to state the phenotype present in a specimen rather than the genotype.

The FBI Laboratory has been investigating the utility of VNTR probes developed by R. White and colleagues at the Howard Hughes Medical Institute, University of Utah Medical Center, Salt Lake City, Utah. Initial data acquired with VNTR probes indicate that this category of probes will offer the detection sensitivity required for forensic body fluid specimens, simplicity of interpretation of RFLP patterns and excellent discrimination potentials. The probes currently being used are designated: YNH24, YNZ22, EFD64.1, EFD64.2, CMM86, CMM101 and YNI10. As an example of detection sensitivity, YNH24 will detect RFLP patterns from as little as 45 ng of genomic DNA. Furthermore, these probes recognize DNA fragments of less than 10,000 bps in size (when digested with RE Hinf or Msp), so concerns about degradation of high molecular weight DNA will be minimized.

Multilocus Repeat Sequences

Recently, Jeffreys *et al.* (1985a) reported that DNA probes derived from noncoding repeat sequences associated with the myoglobin gene locus could simultaneously detect a number of loci within the human genome. The multilocus RFLP patterns that could be generated with two of Jeffreys' probes, named 33.15 and 33.6, can provide a genetic "fingerprint" (Jeffreys *et al.* 1985b, 1986) that is unique to one individual (Figure 9). Essentially, the Jeffreys probes are concurrently detecting allelic variation at multiple loci on several chromosomes. Hybridizations with the Jeffreys probes usually are carried out under conditions of

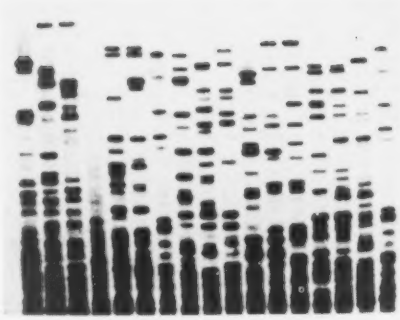

Figure 9. Autoradiogram of restriction fragment length patterns using a multilocus DNA probe (33.15). (Courtesy of Cellmark Diagnostics, Inc.)

low stringency, which permits probe-target duplexes with a higher degree of bp mismatches to remain as hybrids. It has been estimated that the probability that two randomly chosen individuals would exhibit the same multilocus RFLP pattern using these probes is 5×10^{-19} (Gill *et al.* 1985). The significance of these probes to forensic molecular biology is obvious: the individual origin of a specimen could be determined with absolute certainty. A report by Gill *et al.* (1985) indicated that the use of probe 33.15 alone could provide individualization values from 0.2 to 2.4×10^{-11}, depending on the quantity of DNA present in a specimen. Minisatellite fragments have been cloned to yield locus-specific probes (Wong *et al.* 1986; 1987a).

Although the information provided by the use of Jeffreys multilocus probes makes them appear quite attractive for use in forensics, they possess limitations that must be considered. First of all, the Jeffreys probes are proprietary (Cellmark Diagnostics, Germantown, MD) and currently unavailable for purchase. Secondly, the detection sensitivity of these probes is considerably less than that of VNTR probes. One μg of genomic DNA is required for generation of a DNA fingerprint (D. D. Garner, personal communication, 1987), a quantity that will limit their utility with forensic specimens.

Moreover, Werrett (1987) has reported that a 3 cm diameter bloodstain or a 2.5 cm diameter semen stain is necessary for analysis using these probes. The large number of fragment bands revealed by multilocus probes, while advantageous for individualizing, can make interspecimen pattern comparisons extremely difficult, especially when multiple individuals have contributed to a stain specimen. Moreover, these probes recognize sequences common to humans, birds, dogs and cats (Hill 1987; Wetton *et al.* 1987), necessitating an alternate strategy for species determination.

Study of the multilocus probe pattern shown in Figure 9 reveals variations in fragment band intensities. As the amount of DNA in a specimen becomes smaller, some bands may disappear from the autoradiogram altogether with a commensurate reduction in the discrimination value (Gill *et al.* 1987) and possibly lead to interpretational difficulties. A final consideration is from the aspect of DNA degradation. The multilocus probes generate profiles with fragments of sizes between 4,000 and 20,000 bps. As biological samples age and succumb to environmental adversity, the DNA can be degraded so that the recoverable DNA is smaller than 20,000 bps in size.

RESEARCH STRATEGY WITH RFLP PROBES IN THE FBI LABORATORY

The FBI Laboratory's studies into RFLP detection include not only an evaluation of the utility of certain probes, but also the development of strategies that will improve the sensitivity of detection. Since the specimens encountered in forensic cases often are limited in size and may have suffered various degrees of degradation, obtaining RFLP data from the smallest possible quantity of material is of paramount importance. Areas currently being investigated to achieve these goals include: (1) improvements to the DNA extraction techniques, (2) increasing the specific activity of radioactive label in probes, (3) probe labeling methods that do not rely upon radioactive tracers, (4) increasing the resolution of fragments during electrophoresis and (5) improving the efficiency of the Southern blot transfers. An ancillary goal is to simplify the overall technology so that its transfer to state and local laboratories would be facilitated.

AMPLIFICATION OF DNA BY POLYMERASE CHAIN REACTION

The interchange of biological substances between individuals during criminal activities often involves only trace quantities of materials. Small samples of body fluids and human hair (especially telogen hairs) will contain genomic DNA that is qualitatively representative of the donor but quantitatively insufficient for VNTR-RFLP analysis. Moreover, the bulk of the DNA in evidentiary specimens which has been exposed to adverse environmental conditions may well be degraded to a level which is inadequate for RFLP analysis. A method by which short target sequences of DNA might be quantitatively amplified to an analytical level would be extremely useful in the examination of specimens of limited DNA yield. Such a technique has been developed and is called polymerase chain reaction (PCR) (Saiki *et al.* 1985).

Several simple processes characterize the PCR methodology that enables the amplification of subanalytical quantities of DNA: (1) Target DNA is denatured by exposure to an elevated temperature (95^{0}C). This step yields two separate single strands of complementary DNA. (2) Single-stranded short oligonucleotides of known sequence are hybridized (annealed) to regions that flank the target DNA regions of interest. The oligonucleotide is called a primer because upon hybridization to target DNA, it furnishes an initiation site for the activity of DNA polymerase. (3) DNA polymerase, utilizing the four nucleotide triphosphates, will synthesize a new strand of DNA whose sequence will be directed by the template strand to which the primer was attached. The synthesis of the new DNA strand is sometimes referred to as primer elongation. The primers are extended in a 5' to 3' manner. The new strand of DNA will, of course, be the complement of the template strand. Each cycle of these three PCR steps doubles the quantity of target site DNA

initially present. Repeated cycles of the PCR procedure are performed until the desired quantity of DNA is generated. As illustrated in Figure 10, short sequences defined by the 5' terminus of the primers are amplified geometrically so that several hundred thousand copies of the template sequence are produced by 20-25 PCR cycles.

After the PCR cycles have been completed, the amplified DNA can be analyzed by methods that reveal the alleles present at defined loci. Originally, DNA analysis following PCR amplification was carried out by the dot blot procedure using allele specific oligonucleotide probes (ASO) directed at a defined locus (Saiki *et al.* 1986; Erlich 1986). The alleles identified by ASO probes do not vary in size, for the nucleotide sequences are not in the form of multiple repeats but represent point mutation variations. For this reason, no RE digestion or electrophoretic separation of DNA is required in the dot blot method. Dot blot procedures permit the direct binding of target DNA to a membrane as a confined spot. After prehybridization, membrane strips containing the DNA spots are challenged with ASO probes. ASO probes are short oligonucleotides synthesized so as to recognize specific sequences on the target DNA. Under conditions of high stringency, the hybrid formed between the ASO probe and the target DNA cannot tolerate any bp mismatches. Thus, only DNA with the exact complementary sequence to the ASO will be recognized. It should be apparent that an ASO probe is required for each of the allelic forms that can be present at the locus under study.

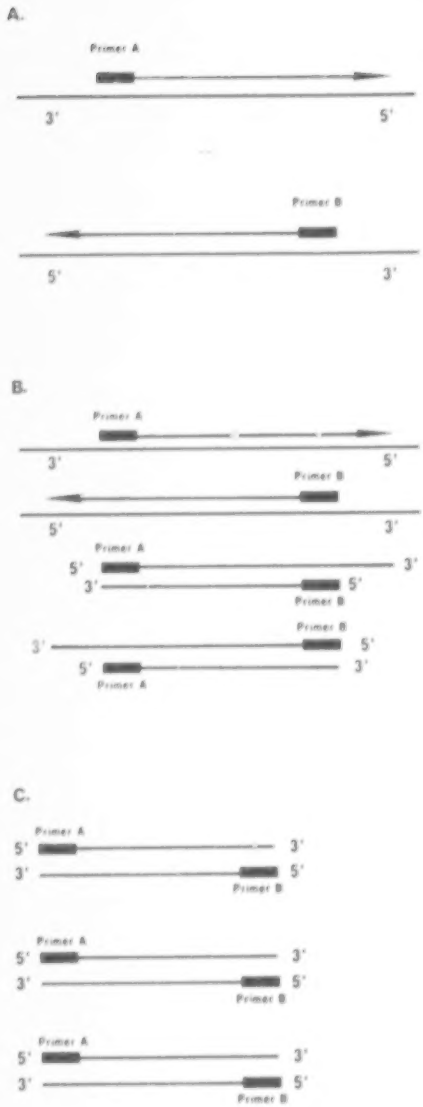

Figure 10. Polymerase chain reaction (PCR) amplification. (A) The first cycle provides a doubling of the target sequence but includes extension beyond it. (B) The second cycle produces two more strands which include the target site and extension beyond, plus two shorter strands whose termini are defined by the 5' end of the primers. (C) In subsequent cycles, the short strands increase exponentially in number, while the longer strands increase linearly in number. Assuming a 100% yield for 25 cycles of PCR for one double-stranded DNA molecule, 50 longer fragments and 67,108,812 short fragments will be produced.

As with RFLP analysis of DNA, the ASO probes must be labeled in some fashion to permit detection of the probe-DNA hybrid. Because PCR generates ample quantities of DNA for analysis, the probes generally need not be tagged with a radioisotope label to determine their presence on the membrane. Erlich (1986) has used ASO probes tagged with the enzyme horseradish peroxidase to signal probe hybridization with target DNA. Upon assay of probed membranes with a chromogenic substrate for peroxidase, the presence of probe binding will be indicated by color development. If the probe has failed to bind to the target, because the specific allele is absent, no color will be seen at the DNA spot on the membrane.

PCR cannot be performed on target DNA loci that are incompletely defined. In order to exploit PCR technology to amplify a polymorphic area of DNA, the nucleotide sequence that defines the region of interest must be known in advance. The sequence of nucleotides that flank the polymorphic sequence has to be known before the primers can be synthesized. Moreover, the sequence that characterizes each of the allelic forms of the DNA must be identified if specific ASO probes are to be made.

Two protein-encoding regions that have been investigated thoroughly are the beta-globin genes (Wong *et al.* 1987b; Ipraim *et al.* 1987) and the genes that direct the synthesis of the HLA-DQ alpha proteins (Erlich *et al.* 1986; Saiki *et al.* 1986; Saiki *et al.* 1985). Both studies used the dot blot test format to genotype/phenotype blood specimens. The polymorphisms that occur at the HLA-DQ alpha locus are of particular interest to forensic scientists. Using six different ASO probes to detect alleles at this locus, 21 phenotypes can be observed in the population. PCR amplification in concert with this ASO probe/dot blot system has been used successfully to type the DNA isolated from a single telogen hair root (Higuchi *et al.* 1986). The FBI Laboratory is investigating the application of PCR methods to the characterization of hair specimens as well as blood and semen stains.

While PCR in concert with dot blot methods potentially can increase the number of evidentiary specimens that can be analyzed for DNA polymorphisms, it should be recognized that ASO probes do not provide the individualization potential that can be achieved with VNTR/multilocus probe-RFLP methods. However, Erlich (1986) reported recently that as many as 3,000 nucleotide base fragments have been successfully amplified by PCR, raising the possibility that RFLP analyses could be carried out on PCR-treated specimens.

Scientists in the FBI Laboratory Forensic Science Research Unit also are evaluating procedures for the amplification of noncoding regions of mitochondrial DNA followed by direct sequencing of the product as a means to individualize forensic specimens. Since there are many more copies of mitochondrial DNA per cell than nuclear DNA, fewer PCR cycles may be required to achieve the analytical threshold. Wrischnik *et al.* (1987) recently reported such an approach for the detection of length mutations in human mitochondrial DNA.

PCR in conjunction with a DNA typing method affords the analyst a greater detection sensitivity than can be achieved by the typing method alone. It obviates the need for labeling of probes with radioisotopes, and it is simple to perform, especially since the process now has been automated. Additionally, PCR can be applied to substantially degraded DNA specimens, for it does not require high molecular weight DNA to be successful.

VALIDATION CONSIDERATIONS

We anticipate that the sophisticated techniques enabling the detection of the polymorphisms that occur in human genomic DNA will be subjected to a thorough legal scrutiny upon their introduction to the courtroom setting. It is the responsibility of those engaged in research directed at the application of recombinant DNA methods to forensic specimens to ensure that these methods have been comprehensively validated and can be applied legitimately to the types of specimens encountered in the crime laboratory. The scientific validity of DNA typing methods per se is not likely to be an issue, for such methods are in widespread

use by diverse groups of scientists. Recombinant DNA methods are employed in basic studies of human genetics, disease susceptibility and anthropological inquiries relating to population migrations. The major focus in the validation of DNA typing methods must be on the legitimacy of their application to human tissue and fluid specimens that were removed from the body in an uncontrolled manner and might have deteriorated prior to collection. The validation of DNA typing methods more than likely will have to follow the same path previously taken by forensic scientists to establish that the analysis of polymorphic proteins was suitable for the genetic characterization of biological evidence.

The Forensic Science Research Unit of the FBI Laboratory has developed a number of validation protocol steps that should be ascended at the research level before DNA typing techniques will be certified for use on case evidence in our laboratory. These steps are described as follows:

1. Perfect the typing methods with fresh body tissues and liquids obtained and stored in a controlled manner.

2. Using specimens taken from donors of known phenotypes/genotypes, evaluate the reproducibility of the techniques both within the laboratory and between unrelated laboratories.

3. Establish population distribution data in different racial groups for the alleles detected by a given RE-DNA probe pair.

4. Prepare dried stains using body fluids from donors of known phenotypes and analyze using the tentatively perfected typing methods.

5. If required, modify the typing methods to ensure that stain specimens exhibit accurate, interpretable and reproducible DNA typing profiles that agree qualitatively with the profiles obtained on liquid specimens.

6. Determine if the polymorphic patterns in dried stains change as a function of storage time.

7. Determine if DNA isolated from various tissues and organs within an individual yields identical typing profiles.

8. Expose laboratory-prepared body fluid stains to a variety of commonly-encountered adventitious substances to assess the impact of these substances on DNA profiles.

9. Examine DNA profiles in nonprobative evidentiary stain materials as examples of stains that are likely to have been exposed to a wide variety of adventitious substances and climatic extremes. Because all possible contaminants and environmental conditions that might affect DNA typing profiles cannot be addressed experimentally, special attention will be directed to a comparison of DNA profiles derived from victim's liquid blood versus victim's blood deposited on typical crime scene substrata.

10. Determine if DNA typing methods designed for use with human specimens detect DNA profiles in fluids and tissues from nonhuman sources.

11. Set up typing methods in the case-working laboratory for onsite validation of methodology.

12. Publish results of experimental studies in peer reviewed journals and present data at scientific meetings. (These mechanisms provide a forum for public criticism of the methodologies.)

13. Train case-working unit personnel in all aspects of the performance of DNA typing methods and interpretation of typing results.

14. Establish formal training sessions for, and engage in collaborative testing procedures with, scientists in state and local forensic science laboratories. This program will not only serve to broaden the base of laboratories capable of performing the analyses, but also will demonstrate that the methods are technologically stable.

This approach should enable research scientists to establish the scientific validity of DNA typing methods as they are applied

to the examination of evidentiary materials and directly address the Frye standard (*Frye* v. *United States* 1923) for admissibility of evidence. The Frye standard will be satisfied if the methodology and validation test results are subjected to scrutiny by the scientific community and found to be generally accepted as reliable by a clear majority of this group. A number of steps in the validation process permit the relevant scientific community to review the methodology and validation data. Publications in peer-reviewed journals, presentations at scientific conferences, workshops and laboratory courses are excellent forums for the exchange of ideas and opinions regarding the merit of DNA typing procedures. During such colloquia, the relevant community will be in a position to decide if the validation data is exact and worthy of acceptance, or if the supporting data is flawed and should be rejected.

A by-product of the validation studies will be answers to a number of important questions regarding the general applicability of DNA typing to forensic case specimens. A list of questions for which answers are sought in this regard would include:

1. What is the minimum size of blood and semen stains that routinely will provide sufficient DNA for RFLP testing?

2. What is the DNA typing efficiency with forensic evidence? If these procedures provide a low conclusive call frequency, the evidence might better be tested for protein and antigen polymorphisms.

3. Should screening tests be carried out on specimens prior to the application of DNA typing methods? Given the time and labor input required to carry out DNA typing procedures, perhaps specimens should be screened by simpler and more rapid procedures to determine if an individual could/could not be eliminated as a body fluid stain donor. Screening procedures of possible benefit in this context would be the venerable ABO antigen tests and one or more of the robust polymorphic protein markers currently used for characterization tests. Another screening test procedure of potential utility would be that directed at detection of specific DNA alleles that code for protein which have been amplified by PCR.

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Inactivation of Human Immunodeficiency Virus (HIV) by Gamma and X-Ray Irradiation in Body Fluids and Forensic Evidence

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In the January 1987 issue of the Crime Laboratory Digest, I published an article entitled "HTLV-III and the Forensic Laboratory." In that article, I mentioned that the FBI Laboratory was conducting research into the feasibility of utilizing gamma and x-ray radiation to inactivate the acquired immunodeficiency syndrome (AIDS) virus in body fluids and forensic evidence. The research study had two goals: (1) to determine the dosage of radiation that was lethal to HIV and (2) to determine if this dosage was deleterious to the biological substances routinely sought in forensic serological analyses. The research was conducted in collaboration with P. S. Sarin of the Laboratory for Tumor Cell Biology at the National Cancer Institute (NCI) and J. C. Humphries of the Ionizing Radiation Division at the National Bureau of Standards (NBS) and has been successfully completed.

Previous research has shown that HIV in concentrated amounts can survive in dried body fluid stains for several days at room temperature (Centers for Disease Control 1987a; Spire *et al.* 1984; Resnick *et al.* 1986). However, in concentrations which would normally be found in persons with AIDS, leading experts generally agree that the possibility of the virus surviving beyond a maximum of 72 hours in a dried state is highly improbable (W. Bond, F. Chermann, P. Markham, L. Resnick, P. Sarin, personal communications). However, Resnick *et al.* (1986) reported that the virus can survive for prolonged periods of time in liquid body fluids (at least 15 days). Recently, several incidents have occurred where HIV-infected liquid blood has been absorbed through broken skin or through the eyes or mucous membranes of health care workers, thus transmitting the virus to these individuals (Centers for Disease Control 1987b). Although the possibility of transmission of HIV to forensic laboratory workers is remote, any available and reasonable means of reducing the risk of possible infection to laboratory workers should be considered.

Our research consisted of first performing all analyses currently conducted in the Serology Unit of the FBI Laboratory on selected samples of liquid and dried blood, semen and saliva. Next, two different bacterial cultures were prepared. Then the previously characterized samples and the cultures were subjected to gamma radiation emitted from a cobalt-60 source in varying amounts from 300,000 rads to one megarad.

Since most forensic laboratories do not have the capability of using gamma radiation, NBS prepared calibration packets for an x-ray instrument in the FBI Laboratory so that levels of gamma radiation could be converted to usable levels of x-ray radiation. Once this instrument was calibrated, the Laboratory for Tumor Cell Biology prepared numerous serial dilutions of live, concentrated HIV (HTLV-III) in cell culture medium.

It was determined that 25,000 rads of x-ray radiation were required to completely inactivate HIV in liquid samples. This amount is a tenfold decrease over that previously reported (Spire *et al.* 1984), and it is believed the reason for this decrease is the less concentrated amounts of virus present in the test samples. It was hypothesized that the earlier reported amounts of radiation required to inactivate the virus (250,000 rads) could be reduced substantially to inactivate samples containing lower concentrations of virus. Serial dilutions were prepared by NCI to the lowest amount of virus that could be detected in body fluids by analyses of reverse transcriptase and cell infectivity. This concentration of virus still exceeds the amount of virus found in a person with fully manifested AIDS. Approximately 25,000 rads of gamma radiation are used to sterilize food

products and sera (Spire *et al.* 1984). This research has shown that using more concentrated levels of virus requires a corresponding increase in irradiation for inactivation of the AIDS virus.

Once the lowest amount of x-ray radiation was determined to completely inactivate the virus, uninfected blood, semen and saliva samples were subjected to the same level of x-ray irradiation.

With the exception of a liquid blood sample irradiated at one megarad of gamma radiation, no denaturation or detrimental effect in any protein of serological importance was observed as a result of either x-ray or gamma irradiation. The liquid blood sample appeared to be hemolyzed, possibly through the formation of free radicals known to occur at high dosage levels of irradiation (Tsoulfanidis 1983), but when dried and analyzed the same as any dried blood stain, no effect on forensic analyses was observed. As expected, the two bacterial cultures were completely sterile after irradiation. When observing the electrophoretic banding patterns in enzyme and serum protein analyses, the irradiation process caused the bands to appear more distinct when compared to the same samples prior to irradiation, possibly due to lack of bacterial degradation.

As a result of the development of this new technique, the FBI Laboratory has altered its policy on the acceptance of evidence from persons with AIDS or exposed to HIV. The FBI Laboratory will accept known AIDS cases; however, the Scientific Analysis Section must be notified telephonically prior to submission when either a suspect or victim has AIDS or is suspected of having AIDS. The FBI Laboratory has implemented a policy wherein liquid blood samples, or any other liquid body fluid from a person known or suspected of having been exposed to HIV, or liquid blood samples from persons in the traditional "high risk categories" such as male homosexuals, prostitutes and intravenous drug users, will be irradiated by x-ray prior to analyses. Dried body fluids which are less than 5 days old from either victims or suspects known to have AIDS will be air dried for a total period of 5 days in a laminar flow biological containment cabinet prior to analyses. Contributors of evidence bearing HIV are reminded that the shipping of this type evidence must be in accordance with Title 42, Code of Federal Regulations, Part 72, which requires the items to be triple wrapped with appropriate warning labels applied.

Regardless of whether or not liquid blood samples have been irradiated, the FBI Laboratory safety policy for handling any liquid body fluid will remain in force. These procedures include the wearing of latex surgical gloves, eye protection, surgical masks and laboratory coats.

The detailed results of this research project are being compiled and will be published in the near future. The use of ionizing radiation is dangerous if not properly managed. Only qualified and trained/certified x-ray technicians may operate these devices, with proper shielding, safety and radiation monitoring procedures in effect and in accordance with the Nuclear Regulatory Commission policies. Any other use of this technique may result in a lethal dosage of radiation to laboratory personnel.

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Meeting Announcements

REGIONAL FORENSIC SCIENCE ASSOCIATION MEETINGS

The Midwestern Association of Forensic Scientists (MAFS) is sponsoring its fifth Bloodspatter Workshop at Silver Lake Camp, Minneapolis, Minnesota from April 25-29, 1988.

For further information contact MAFS Workshop Co-chairmen Terry Laber or Bart Epstein, BCA Forensic Science Laboratory, 1246 University Avenue, St. Paul, Minnesota 55104 (telephone: 612-642-0700).

The Midwestern Association of Forensic Scientists (MAFS) will hold Spring Workshops on the ATF Accelerant Detection Class and Archaeology, Anthropology and Odontology of Burial Sites at the Sheraton Inn in Madison, Wisconsin from May 2-6, 1988.

For further information, contact Michael A. Haas, Workshop Chairman, Crime Laboratory Bureau - Madison, 4706 University Avenue, Madison, Wisconsin 53705-2157 (telephone: 608-266-2031).

The Mid-Atlantic Association of Forensic Scientists (MAAFS) will hold its 1988 Annual Meeting at the Ramada Oceanside Tower in Virginia Beach, Virginia from May 5-6, 1988.

For further information, contact Jo Ann Kern, NIS Regional Forensic Laboratory, Norfolk Naval Station, Norfolk, Virginia 23511-6493 (telephone: 804-444-8615) or Robert Campbell, Tidewater Regional Forensic Laboratory, 401-A Colley Avenue, Norfolk, Virginia 23507-1966 (telephone: 804-623-8327).

The Southern Association of Forensic Scientists (SAFS) will hold its Spring 1988 Meeting at the Peabody Hotel in Memphis, Tennessee from May 5-7, 1988.

Scientific papers are requested for presentation at this meeting, which will include sessions on toxicology, serology, solid dosage, pathology/biology, criminalistics and firearms. In addition four workshops are scheduled as well as a presentation on DNA polymorphisms.

For further information, contact Steve Nichols or Paulette Sutton, University of Tennessee, Toxicology Laboratory, 3 North Dunlap Street, Memphis, Tennessee 38163 (telephone: 901-528-6355).

The California Association of Criminalists (CAC) will hold its Spring 1988 Seminar at the Marriott Marina Hotel in Berkeley, California from May 19-21, 1988.

For further information, contact Charles Morton, Institute of Forensic Sciences, Criminalistics Laboratory, 2945 Webster Street, Oakland, California 94609 (telephone: 415-451-0767).

The Southwestern Association of Forensic Document Examiners will hold its Spring 1988 Questioned Documents Seminar at Karl's Silver Club, Sparks, Nevada from May 27-30, 1988.

For further information, contact Floyd Whiting, Washoe County Sheriff's Department, Crime Laboratory, P.O. Box 2915, Reno, Nevada (telephone: 702-785-6211).

The Midwest Regional AOAC will hold a meeting at the Holiday Inn West in Columbia, Missouri from June 20-22, 1988. A session on forensic analysis is planned.

For additional information on the forensic analysis session, contact Robert Briner, SEMO Regional Crime Laboratory, Southeast Missouri State University, Cape Girardeau, Missouri 63701 (telephone: 314-651-2221).

The Southern Association of Forensic Scientists (SAFS) will hold its Fall 1988 meeting at the Adams Mark Caribbean Gulf Resort in Clearwater Beach, Florida from September 7-10 1988.

For further information, contact Karen Cudlin, Pinellas County Sheriff's Department, Forensic Laboratory (telephone: 813-584-7345).

The Midwestern Association of Forensic Scientists (MAFS) will hold its Fall 1988 Meeting at the Sheraton Park Place Hotel, Minneapolis, Minnesota from October 3-7, 1988. This meeting will be hosted by the Minnesota BCA Forensic Science Laboratory and will include workshops in serology, chemistry, toxicology, questioned documents and latent prints.

For further information, contact Laura Nelson or Dan Bergman, BCA Forensic Science Laboratory, 1246 University Avenue, St. Paul, Minnesota 55104 (telephone: 612-642-0700).

The 14th Annual Meeting of the Northeastern Association of Forensic Scientists (NEAFS) will be held at the Mystic Hilton in Mystic, Connecticut from October 21-22, 1988.

For further information, contact Steve Sottolano, Drug Enforcement Administration, Northeast Laboratory, 555 West 57th Street, Suite 1886, New York, New York 10019 (telephone: 212-399-5137).

NATIONAL FORENSIC SCIENCE ASSOCIATION MEETINGS

The 19th Annual Seminar of the Association of Firearm and Toolmark Examiners (AFTE) will be held at the Nordic Hills Resort and Conference Center in Itasca, Illinois from June 13-17, 1988.

Conference registrations are now being accepted. The registration fee is $65 for AFTE members and $75 for other registrants, to be paid by May 30, 1988. Late registration after this date will cost and additional $10. Registrants also have the option of paying a daily fee of $20 to attend the conference.

For further information on the conference, contact Program Chairman Richard T. Vaughan, DuPage County Sheriff's Crime Laboratory, 501 North County Farm Road, Wheaton, Illinois 60187 (telephone: 312-682-7614).

The Bureau of Alcohol, Tobacco and Firearms (ATF) will conduct a course in the Systematic Analysis of Low Explosives at ATF's National Laboratory Center (NLC) in Rockville, Maryland from June 13-17, 1988. The course is offered to state and local forensic chemists, and enrollment is limited to ten students.

This 1-week school is designed for forensic chemists who perform or will be performing laboratory analysis of explosive debris. It will emphasize practical skills in the recovery and identification of low explosives and their residues. Topics to be covered include:

a) recognizing low explosives based on blast damage and explosive effects;

b) gathering and documenting evidence at a bomb crime scene;

c) describing the chemical composition of low explosives and post-blast residues;

d) microscopically recognizing explosives in explosion debris;

e) analyzing bomb debris chemically (using extraction procedures, spot tests, organic and inorganic thin layer chromatography and infrared spectrophotometry);

f) developing an appropriate chemical analysis scheme for many of the low explosives frequently encountered; and

g) recognizing post-blast bomb components.

Since individual examiner experience varies, each selected applicant will be sent a short self-test and reading material on explosives/explosions. This test must be completed and returned to the ATF National Laboratory Center by a specified date. Failure to return the test will result in forfeiture of the applicant's class slot.

There will be no tuition, registration or course material fees. The ATF Laboratory will arrange a group rate ($66 per night, including tax) for all selected students at a hotel convenient to the NLC. All transportation, lodging and per diem costs must be paid by the local department or individual. The U. S. Government per diem rate for the Washington D. C. metropolitan area is $112 per day. Attendees should budget accordingly.

To obtain an application for the school, contact Rick Tontarski or Rick Strobel, ATF NLC, 1401 Research Boulevard, Rockville, Maryland 20850 (telephone: 202-294-0420).

The 16th Annual Meeting of the American Society of Crime Laboratory Directors (ASCLD) will be held at the National Emergency Training Center in Emmitsburg, Maryland from September 6-9, 1988.

For further information contact Dorothy Gordimer (telephone: 201-654-3000), Michelle Kestler (telephone: 213-485-2544), Richard Tanton (telephone: 305-471-2220) or Brad Telyea (telephone: 503-229-5017).

The 41st Annual Meeting of the American Academy of Forensic Sciences (AAFS) will be held at the Riviera Hotel in Las Vegas, Nevada from February 20-25, 1989.

For further information, contact AAFS, 225 South Academy Boulevard, Colorado Springs, Colorado 80910 (telephone: 303-596-6006).

The 42th Annual Meeting of the American Academy of Forensic Sciences (AAFS) will be held at the Clarion Hotel in Cincinnati, Ohio from February 19-24, 1990.

For further information, contact AAFS, 225 South Academy Boulevard, Colorado Springs, Colorado 80910 (telephone: 303-596-6006).

INTERNATIONAL FORENSIC SCIENCE ASSOCIATION MEETINGS

The 10th Australian and International Forensic Science Symposium will be held at the Gateway Hotel in Brisbane, Australia from May 23-27, 1988.

The theme of this year's symposium is "Forensic Evidence in the Age of Technology", and it will include sessions in all fields of forensic science. The symposium coincides with World Expo '88 which is also being held in Brisbane from April through October, 1988.

For further information about the symposium, contact Neil Raward, Scientific Section, Queensland Police Department, GPO Box 1440, Brisbane Q 4001, Australia.

The Canadian Society of Forensic Science will hold its 1988 Annual Conference at the Westbury Hotel in Toronto, Canada from October 19-21, 1988.

There will be workshops and plenary sessions on computer applications, DNA, color theory, pharmacokinetics and impaired driving, as well as scientific sessions.

For further information, contact the Canadian Society of Forensic Science, 2660 Southvale Crescent, Suite 215, Ottawa, Ontario, Canada K1B 4W5 (telephone: 613-731-2096).

The 13th International Congress of the International Society for Forensic Hemogenetics will be held in New Orleans, Louisiana from October 18-20, 1989.

For further information contact Dr. Herbert Polesky, Memorial Blood Center, 2304 Park Avenue South, Minneapolis, Minnesota 55404.

Instructions for Submitting Articles

In order to facilitate rapid publication, submissions should conform to the following:

1. Manuscripts should be typed, double-spaced, on 8 1/2" x 11" paper. Submit three copies of the manuscript (one of which must be the original with glossy photographs).

2. Tables should be as simple as possible. Each table should be typed on a separate sheet of paper and should include a heading.

3. Graphs and diagrams should be submitted as sharp photographs on white glossy paper.

4. References in the body of the paper should refer to the author's (authors') name(s) and the date of publication. For example:

   According to Doe and Smith (1972)....

   ....has been discussed in the literature (Doe and Smith 1972; Weinburger et al. 1984; Jones 1981).

5. References at the end of the paper should appear in alphabetical order. Journal references should include the author's (authors') name(s), date of publication, title of article, journal name, volume number, and the beginning and ending pages of the article. Please use the following format:

   Davis, H. E. and Jones, B. A. (1978). Seasonal variations in plasma hormones, Biol. Reprod. 88:271-273.

   List articles or chapters within a book as follows:

   deHaseth, J. A. (1982). Fourier transform infrared spectrometry. In: Fourier, Hadamard and Hilbert Transformations in Chemistry, (Marshall, A. G., ed.) Plenum Press, New York, pp. 387-420.

6. Technical papers should be written in an Introduction, Materials and Methods, and Results and Discussion format.

7. When reference is made to a particular product, state the manufacturer of the product and the city and state of the manufacturer's headquarters. For example:

   Isotactic PS (Dow Chemical, Midland, MI)....

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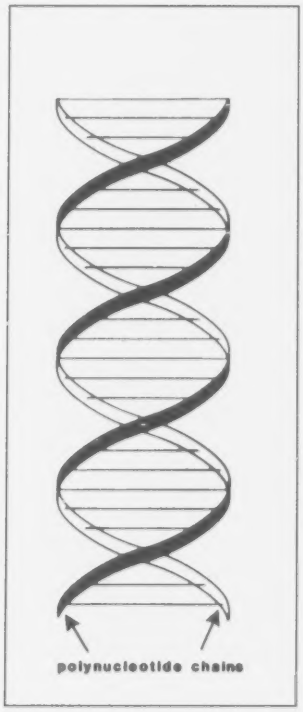

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The Crime Laboratory Digest is published quarterly by the FBI Laboratory in cooperation with the American Society of Crime Laboratory Directors (ASCLD). It is intended to serve as a rapid means of communication between crime laboratories, permitting information of interest and value to be disseminated among crime laboratory scientists.

Inclusion of an article in the Crime Laboratory Digest in no way represents an endorsement or recommendation of any part of that article by the Federal Government, the Department of Justice or the FBI. Contributing authors assume total responsibility for the contents and accuracy of their submission. Questions or requests concerning an article should be directed to the contributing agency.

All submissions are subject to editorial review in accordance with the editorial policy established by the FBI Laboratory and ASCLD. The editorial staff of the Crime Laboratory Digest reserves the right to edit all articles for style, grammer and punctuation. Comments and letters to the editor are encouraged and will be published when appropriate and as space permits. These should be forwarded to:

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Message from the Assistant Director in Charge of the FBI Laboratory

This Supplemental Issue of the Crime Laboratory Digest is provided as a means to transmit specific information to you which may be useful in your planning cycle for the implementation of DNA testing methods. A limited printing of this Supplemental Issue was mailed to approximately 300 crime laboratories in the United States in early April.

The FBI is making steady progress in its DNA research program and in its effort to transfer this important technology to the crime laboratory community. In early April, invitations were extended to directors of the laboratory systems throughout the United States to nominate individuals to participate in the Visiting Scientist Program at the Forensic Science Research and Training Center (FSRTC). This 4-month program is designed to provide the technical resources to address the validity and reliability issues associated with DNA testing as quickly as possible. Upon completion of the collaborative research project, participants will have developed a proficiency in the test methods and gained valuable experience to apply to DNA implementation efforts in their individual state and local laboratories.

To assure maximum benefits from the Visiting Scientist Program, we requested that sponsoring laboratories be fully committed to DNA implementation as indicated by appropriate equipment inventories and appropriate authority to use the radioactive isotopes employed in the DNA probes. The information in this issue is intended to help further clarify these requirements. An attempt was made to express these requirements in "generic" terms, that is, in terms that can be readily modified and tailored to an individual laboratory's needs, based upon existing facilities, examination workload and anticipated personnel to be committed to DNA testing. They should be considered as a guide for planning purposes and not necessarily a "shopping list." You are encouraged to consult with individuals experienced in DNA test procedures and discuss your specific requirements.

For your additional information, a curriculum is being developed for a DNA training course for state and local laboratory personnel which we expect to begin offering in the late Fall of this year. You will be advised as soon as the program is finalized and student applications can be submitted. The program will recommend academic background criteria for student candidates, including recent participation in a college level course on molecular biology as well as a bibliography of recommended readings to prepare for the course. A brief description of the recommended course content and an abbreviated bibliography is in this issue.

From 5/31-6/2/88, a seminar on DNA technology is being sponsored at the FSRTC. Attendance is limited at this conference due to housing constraints at the FBI Academy facility. Participants will include key forensic and medical researchers from academia, the private sector and the international crime laboratory community. Two important topics to be addressed at this meeting are the establishment of DNA standards within the forensic science community and automated data files containing DNA identification profile information. Some states have already passed or proposed legislation which provides for the collection of blood standards from convicted sex offenders. A data system containing DNA profile information from semen or other biological evidence recovered from the victim or scene of a sexual assault would permit cross comparisons to link similar crimes within the same jurisdiction or among several jurisdictions. Clearly, it would be advantageous to law enforcement on a national scale to coordinate the development of such systems with the establishment of appropriate controls and standards to permit the effective exchange of DNA identification profiles. To accomplish this, the community must agree upon standards which provide a common language and thereby facilitate the exchange of critical investigative information and, at the same time, build flexibility into the system to accommodate changes as this technology continues to evolve. The success of this effort will depend heavily on a

strong professional commitment to the needs of law enforcement along with a spirit of cooperation and mutual support within the forensic community. A report will be prepared on the events of the May-June seminar for presentation at the Las Vegas annual meeting of the American Academy of Forensic Sciences. I hope this information is useful to you, and I encourage any comments or questions you may have. It is our goal to identify the means for the full realization of DNA technology in the forensic community and to facilitate its implementation in any way possible.

ROGER T. CASTONGUAY

A Primer on the Methods Used in the Typing of DNA

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STRUCTURE OF DNA

Deoxyribonucleic acid (DNA) is an organic substance found primarily in the chromosomes that are structures within the nuclei of cells. The DNA within any chromosome is composed of two strands, wound about each other in a helical fashion. Each DNA strand is a polymer, composed of molecules, called nucleotides. Four different nucleotides are found in DNA, deoxyadenosine monophosphate (A), thymidine monophosphate (T), deoxycytidine monophosphate(C), and deoxyguanosine monophosphate (G). It is more convenient for the following discussions to refer to the nucleotides solely by their letters, A, C, G, and T.

A single strand of chromosomal DNA is a chain composed of hundreds of thousands of these letters. Because the order of the four letters can vary from one position to the next in the chain, an almost infinite variety of letter sequences can be developed. Chromosomal DNA, as was stated, is composed of two strands, and these two strands associate with each other in a certain way that is governed by the chemical properties of the letters in each strand. The letter A in one strand will associate only with the letter T in the other strand. Likewise, the letter C in one strand will only associate with G in the other strand. A short sequence of double-stranded chromosomal DNA might look like this:

-A-C-G-T-A-A-A-A-G-T-T-C-C-
-T-G-C-A-T-T-T-T-C-A-A-G-G-

If the sequence of letters in one strand is known, the sequence in the other strand can be deduced. The association of one DNA strand with another is not strong. If the DNA is heated to near the boiling point of water, the strands will separate. When the DNA cools, the two strands will line up in the proper way and reestablish their association. This property of DNA is important in understanding how certain sequences are detected in the DNA.

CHROMOSOMES AND HEREDITY

The nucleus of every cell in the human body contains 23 pairs of chromosomes for a total of 46. The exceptions are the sperm cell and the female ovum (egg cell), which contain only 23, unpaired, chromosomes each. When a sperm cell fertilizes an ovum, the cell that results (zygote) will possess 46 chromosomes, 23 from the father and 23 from the mother. Since the chromosomes are the cellular repositories of DNA, one-half of the DNA in a person is of paternal origin and one-half is of maternal origin.

REPETITIVE SEQUENCES IN DNA

Many of the letter sequences in chromosomal DNA code for the production of proteins that are required for the development and maintenance of life. These functional sequences are called genes, and always are inherited in pairs. One member of a gene pair is received from each parent.

All the letter sequences in DNA do not code for the production of proteins. Within the letter sequences that intervene between the protein coding regions can be found short sequences of letters that are repeated multiple times. Figure 1 illustrates an imaginary repeat sequence in DNA. The two double-stranded sections of DNA shown represent the multiple repeat regions from each member of a chromosomal pair. The DNA from one chromosome contains six repeats of the sequence -C-A-T-; and the DNA from its partner chromosome contains three repeats of the same sequence. The six repeat sequence was inherited from one parent and the three repeat sequence from the individual's other parent.

-G-A-T-A-C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-G-G-A-T-A-T-G-G-
-C-T-A-T-G-T-A-G-T-A-G-T-A-G-T-A-G-T-A-G-T-A-C-C-T-A-T-A-C-C-

-G-A-T-A-C-A-T-C-A-T-C-A-T-G-G-A-T-A-T-G-G-A-A-G-T-T-T-A-C-G-
-C-T-A-T-G-T-A-G-T-A-G-T-A-C-C-T-A-T-A-C-C-T-T-C-A-A-A-T-G-C-

Figure 1. Two double-stranded DNA sections.

RESTRICTION FRAGMENT LENGTH POLYMORPHISMS

The presence in DNA of multiple repeats of letter sequences is the foundation on which rests one of the methods by which body fluid and tissue specimens can be genetically characterized. The characterization is based on a determination of the lengths of the repeated sequences harbored in specific areas of an individual's DNA. The length of a section of DNA that contains the repeated sequences is a direct function of the number of times the sequence is repeated. To determine the length of the repeat sequence present in each of the chromosomes, the repeating units must be cut out of the DNA strands and separated on the basis of their length. The repeat sequences are removed from the DNA strands through the use of special enzymes called **restriction endonucleases**. These enzymes, which are obtained from bacteria, will cut through both strands of DNA only when a certain recognition sequence of DNA letters is present. For the strands of DNA shown above, assume that the endonuclease will cut DNA wherever the sequence -G-A-T- occurs. Because the -G-A-T- sequence appears on both sides of the repeated sequence, these sequences will be freed in their entirety from the long strands of DNA upon enzymatic digestion. The repeated sequences that have been released by restriction endonuclease digestion (Figure 2) are termed restriction fragments. Length differences in the DNA that can be detected following restriction digestion are termed restriction fragment length polymorphisms (RFLP).

-A-C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-G-
-T-G-T-A-G-T-A-G-T-A-G-T-A-G-T-A-G-T-A-C-

-A-C-A-T-C-A-T-C-A-T-G-
-T-G-T-A-G-T-A-G-T-A-C-

Figure 2. Fragments of DNA released by enzyme digestion.

Before the fragment lengths can be measured, the fragments must be separated from one another on the basis of their size. The separation of DNA fragments by size is accomplished by a technique known as electrophoresis. The restriction-digested DNA is placed into a gel that acts like a molecular sieve when the DNA fragments are pulled through it by an electrical field. During electrophoresis, the smaller fragments will move farther through the gel than can the larger fragments.

The behavior of the restriction fragments during electrophoresis is shown in Figure 3. The greater length of the larger fragment causes it to pass more slowly through the sieving gel than the smaller fragment. After about 16-20 hours of electrophoresis, the shorter fragment will have moved farther down the gel than the longer fragment.

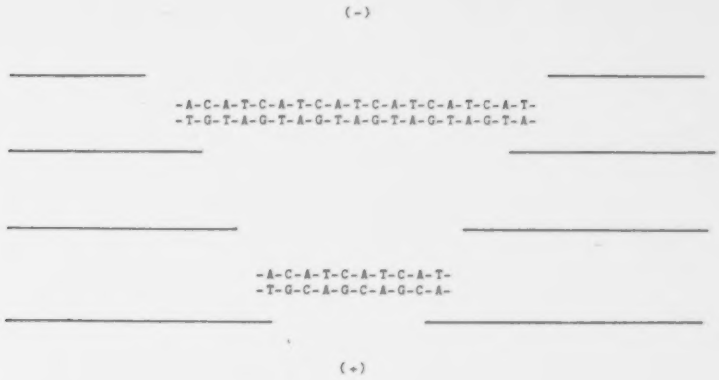

Figure 3. Illustration of sieving properties of an electrophoretic gel.

Once the fragments of DNA have been physically separated, the remaining steps in the analysis are directed at an identification of the sizes of the fragments. The size identification steps cannot be done readily if the DNA fragments remain in the separation gel, so they are transferred to a nylon membrane in exactly the same array as they existed in the gel. This transfer is known as Southern blotting and is named for the developer of the technique.

After transfer to the nylon membrane, the fragment locations on the membrane are established. This is done by using a DNA probe. A DNA probe is a short sequence that is complementary to the region of DNA that one wishes to detect. In the case of the C-A-T sequences, the probe must be composed of the letters G-T-A because this letter sequence is the complement of C-A-T. DNA probes can be tagged with radioactivity to facilitate their detection later in the analysis.

The membrane, with its array of DNA fragments, is immersed overnight in a solution that contains the radioactive probe of sequence G-T-A. During this time, the probe molecules will match up with the areas of the DNA fragments that contain the complementary sequence of letters. For the two fragments shown in Figure 3, there will be six probe molecules bound to the large fragment and three probe molecules bound to the smaller fragment. After this procedure takes place, which is called hybridization, the membrane is washed thoroughly to remove any unbound probe.

The final step is directed at determining which areas of the membrane contain radioactivity. The membrane is covered with a sheet of unexposed X-ray film and stored from one to seven days at a very low temperature. During this storage, the radioactive particles expose the X-ray film at the exact location of the DNA fragments that have hybridized with the probe molecules. When the X-ray film is developed, the locations of the fragments that have hybridized with the probe are seen as lines on the film (Figure 4).

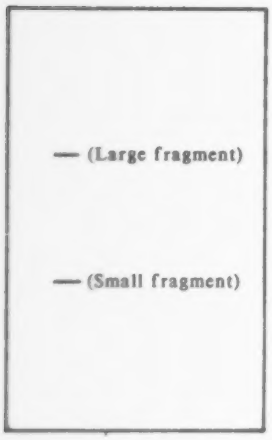

Figure 4. Appearance of fragments on X-ray film.

The actual size of each of the fragments is calculated by reference to standards of DNA of known size that are run in parallel with the test specimens. The determination of fragment sizes can be carried out by image analysis techniques.

The fragment pattern shown in Figure 4 is illustrative of the result when a single locus DNA probe is used. Single locus probes are used to detect repetitive sequences that occur at a single location in the DNA from one pair of chromosomes. The maximum number of bands that should be seen on the X-ray film is two (one band is derived from each chromosome). The appearance of more than two bands on the X-ray film suggests that body fluids from more than a single individual were present in the test specimen.

The number of different sized repetitive sequences that occur in the human population that are detected by a given single locus probe is limited, and varies with the DNA probe being used as well as the restriction endonuclease used to cut the DNA strands. One of the DNA probes in use by the FBI Laboratory enables the detection of more than 50 different sized repetitive sequences in the population. Because at most, one individual can possess only two different sized sequences in his/her DNA (when using a given probe), the number of different combinations of size sequences that can be present is large. As the number of combinations becomes large, the chance of two individuals having the same two fragment sizes becomes correspondingly small. Although the use of a solitary single locus probe can reduce considerably the number of potential contributors of a body fluid, additional DNA probes must be applied to the resolved fragments to enable absolute individualization of the specimen source.

To genetically characterize a pair of specimens to the extent that it can be stated that they could have originated from but a single individual, requires the application of several single locus DNA probes, each of which recognizes a different, and genetically unrelated, series of repeated DNA sequences. For example, assume that five different DNA probes will be used to compare the DNA of two specimens. Further assume that the likelihood of two people sharing the same size repeat DNA sequences for each of the probes is 1 in 100. For the five probes, the net likelihood that the two people will match is 1 in 100 X 100 X 100 X 100 X 100 = 1 in 10,000,000,000. Clearly, the use of five such probes would eliminate more potential donors than are living on earth.

Another type of DNA probe that can be used to detect restriction fragments of different sizes is known as a multilocus probe. While similar to single locus DNA probes in that they detect repeated sequences in DNA fragments, they differ in that multilocus probes simultaneously detect DNA fragments from multiple regions of multiple chromosomes. Dr. Alec Jeffreys was the first to recognize the utility of multilocus probes. He coined the term "DNA fingerprinting" to describe the application of multilocus probes to the genetic characterization of forensic specimens.

The procedures for using multilocus probes are essentially the same as for single locus probes. Chromosomal DNA is isolated, cut with a restriction endonuclease, subjected to electrophoresis, and the fragment array is tested with a DNA probe. The band patterns that develop on the X-ray film after using multilocus probes are somewhat

more complicated to interpret than for single locus probes. It would not be uncommon to have more than 20 fragment bands appear on the film. In fact, when using multilocus probes with fragmented DNA, one does not know in advance how many bands will appear. For this reason, it is difficult to recognize a mixture of body fluids from two or more individuals. Because the multilocus probe recognizes sequences from multiple sites simultaneously, the chances of two individuals at random matching at all the band positions is enormously low. It is conceivable that by the use of only a single multilocus probe one would be able to individualize a body fluid specimen.

STAIN QUALITY/QUANTITY AFFECTS SUCCESS OF RFLP ANALYSIS

Controlled studies in the laboratory have shown that RFLP patterns can be discerned when the analysis was begun with bloodstains as small as 5 uL and semen stains as small as 1 uL (one uL is about 1/50 the volume of one drop from an eye dropper). Because environmental vagaries can have an appreciable effect on body fluid stains, the recovery of DNA from actual case evidence stains will undoubtedly be less than that seen with stains prepared for research studies.

The genetic characterization of forensic biological materials by RFLP analysis requires that the DNA in the cells not be fragmented prior to the initiation of the analysis. Fragmentation of the DNA must be carried out in a controlled manner by digestion with restriction endonucleases. If the chromosomal DNA strands have been broken because the tissue or body fluid stains have been adversely affected by the environment, RFLP analysis may not be possible. A list of potential environmental antagonists of DNA would have to include: sunlight, bacterial growth, heat, moisture, and chemicals. Scientists who perform these procedures will, early in the analysis, conduct a test on the DNA isolated from the stain that measures the extent of DNA deterioration. If this test indicates that extensive deterioration has occurred, the remaining steps in the procedure will not be carried out.

TYPING OF SUBANALYTICAL QUANTITIES OF DNA

The interchange of biological substances between individuals during criminal activities often involves only trace quantities of materials. Small samples of body fluids and human hair (especially telogen hair) will contain DNA that is qualitatively representative of the donor but is quantitatively inadequate for RFLP analysis. An alternative procedure can be carried out on these subanalytical quantities of DNA to enable the genetic characterization of such specimens; although not to the same extent as is possible by RFLP analysis. This technique is called polymerase chain reaction (PCR). The PCR method repetitively reproduces copies of a sequence of DNA until the quantity of DNA copies is sufficient for testing with a special category of DNA probes.

The mechanism by which PCR effects the duplication of the DNA specimen is composed of three steps. For example, assume that the following sequence of DNA is to be increased in quantity by the PCR procedure.

-A-T-T-C-G-G-C-A-C-T-C-C-G-A-A-
-T-A-A-G-C-C-G-T-G-A-G-G-C-T-T-

First, the two strands of DNA must be separated by raising the temperature to about 95^{0}C.

-A-T-T-C-G-G-C-A-C-T-C-C-G-A-A-

-T-A-A-G-C-C-G-T-G-A-G-G-C-T-T-

Second, short pieces of laboratory synthesized DNA, called primers, are permitted to hybidize to each DNA strand as the temperature is lowered. The sequence of DNA letters that immediately precede the region that is to be amplified must be known in order to synthesize the primers.

-A-T-T-C-G-G-C-A-C-T-C-C-G-A-A-
-T-A-A-G-

-T-A-A-G-C-C-G-T-G-A-G-G-C-T-T-
-C-G-A-A-

The third step is called primer elongation and is effected by DNA polymerase and the four letters (A, C, G, T) that compose DNA. The DNA polymerase will start at the end of the primer and, by using the four letters, synthesize complementary copies of each of the two strands.

-A-T-T-C-G-C-A-C-T-C-C-G-A-A-
-T-A-A-G-C-G-T-G-A-G-G-C-T-T-

-T-A-A-G-C-G-T-G-A-G-G-C-T-T-
-A-T-T-C-G-C-A-C-T-C-C-G-A-A-

After the first round of synthesis has been completed, the temperature of the reaction is once again raised to 95^{0}C to separate the DNA strands. Primers again hybridize with what are now four strands and the temperature reduced so that the DNA polymerase can reinitiate DNA synthesis starting at the primer sites. Each cycle of PCR doubles the quantity of DNA that was present at the beginning of the cycle. Thus, the PCR procedure increases the quantity of DNA strands in a geometric manner (that is, 2, 4, 8, 16, 32, etc). After approximately 20-25 cycles, sufficient DNA should have been produced to enable the testing of the sample with special DNA probes.

PCR generated DNA has been tested with probes that have been produced to hybridize with the regions of DNA that code for the synthesis of cellular transplantation antigens called HLA-DQ. Because the number of HLA-DQ antigens that occur in the population is not large, the degree to which a DNA specimen can be genetically characterized with these probes is considerably less than that achievable by RFLP analysis. However, the typing of PCR-generated DNA with this type of probe is less complicated to carry out than the RFLP technique. Because the differences in the HLA-DQ antigens are due to different arrangements of a fixed number of DNA letters rather than due to different lengths of the same sequence, an electrophoretic separation of DNA is unnecessary. Instead, replicate volumes of PCR-generated DNA are spotted onto a nylon membrane and allowed to dry. The replicate spots are tested with a series of HLA-DQ probes that are individually specific for each of the different HLA-DQ sequences. DNA from one individual will demonstrate binding with a maximum of two different HLA-DQ probes if the person carries different DNA codes for these antigen sequences on each chromosome. If only one probe of the series hybridizes with the DNA, the individual probably carries the same HLA-DQ sequence in the DNA of both chromosomes.

Probes used to test for the HLA-DQ DNA sequences usually are not labeled with radioisotopes as an aid to their detection. The probes can be linked to an enzyme whose catalytic activity produces a color at the location where the probe has been bound.

DIRECT SEQUENCING OF THE DNA CODE

A third approach by which an individual's DNA can be genetically characterized is by determining the sequence of DNA letters in a region of DNA in which the order of the letters varies greatly among individuals. Such a region appears to be present in the DNA that is found in the energy producing centers of the cell, which are called mitochondria. The sequence of DNA letters in a portion of the double stranded mitochondrial DNA appears to be highly variable, and can potentially serve as a means to genetically characterize the individual source of DNA.

The direct DNA sequencing procedure bears a similarity to PCR in that primers must be attached to the separated DNA strands and DNA polymerase is present to synthesize the complementary strands of DNA. However, the primers are fashioned so that they attach to only one of the two strands and in addition to the four DNA letters that serve as building blocks for the new strands, specially synthesized derivatives of the four letters are present also. Because each of the normal DNA letters is present in the reaction solution as well as the derivative letters, the potential exists for the DNA polymerase to incorporate a derivative letter into the synthesized strand instead of the normal letter. If a derivative letter is inserted, the synthesis of the strand ceases at that point. The termination of the growing DNA strands will occur each time a derivative letter is incorporated. The ratio

of normal letters to derivatized letters is such that the synthesis of some strands will be terminated at each letter position in the strand, while other strands will not be terminated because normal letters have been incorporated.

At the end of the synthetic phase of the procedure, there will be present in the reaction tube, a series of synthesized DNA fragments that differ in length by one letter, with the last letter in each fragment being a derivative.

The next step in the procedure is the electrophoretic separation of the fragments based on size. The fastest moving fragments (shortest) will be those whose synthesis was terminated when the DNA polymerase incorporated the first letter into the strand and did so with a derivative letter. The slowest in electrophoretic migration will be those fragments (longest) that were not terminated until the last letter in the strand was reached and a derivative was incorporated. The sizes of the fragments are determined as the electrophoresis is in progress. As the fragments pass a fixed point in the electrophoretic gel, a scanning device registers fragment passage. In one type of DNA sequencer, each of the derivative DNA letters is labeled with a distinctive fluorescent molecule, enabling the sequencer electronics to determine which derivative letter had been incorporated into that particular fragment. After all the fragments have passed the detector, the complete sequence of letters will have been determined. If necessary, the sequence of the complementary strand can be determined in a similar fashion (using the appropriate primer) as a check on the authenticity of the letter sequence that has been determined in the first strand.

Expenses Associated With DNA Typing Methods

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A. RESTRICTION FRAGMENT LENGTH POLYMORPHISMS

I. Equipment

The conduct of DNA typing tests through analysis of restriction fragment length polymorphisms (RFLP) does not require any sophisticated items of equipment. Laboratories that currently perform the genetic typing of body fluids by electrophoretic methods probably have a number of the items listed in Table 1. Items in the list marked by an asterisk * would be considered desirable, but not essential, for the conduct of DNA typing.

Other items that are needed such as a pH meter, stirring hotplate(s), balances, glassware, aliquot mixer(s), refrigerator(s) and a source of ice should be in the inventory of every laboratory.

Several types of laboratory hoods should be available in the DNA laboratory. A hood should be dedicated solely to the preparation of radioactively-labeled DNA probes. A fume hood should be available for the handling of organic solvents such as phenol and chloroform. Finally, consideration should be given to the purchase of a laminar flow hood that will protect personnel from biohazards during the initial removal of stains from evidentiary items. The cost of these hoods will range from $3,000 to $6,000 each.

The purified water that is available in the laboratory for solution preparation can be critical to the success of the analysis. At a minimum, water must be passed through a multi-stage ion exchange process followed by distillation, or vice-versa. Assuming that the laboratory is currently using distilled water, the cost of a deionizing system will be approximately $3,000.

Finally, a photographic darkroom is necessary for loading and processing of X-ray film.

II. Supplies

The typing of DNA by RFLP analysis requires a considerable inventory of consumable chemical reagents and supplies. Some of the biochemical reagents are quite expensive (for example, restriction enzymes and radioisotopes). Other items such as microcentrifuge tubes and micropipette tips, while not expensive individually, are used in large numbers. Based on current rates of utilization in the Forensic Science Research Unit, the supply/reagent costs per specimen analyzed is approximately $12. **This value does not include the cost of the DNA probe.** Presently there is no commercial vendor of the DNA probes that are under consideration for incorporation into the FBI Laboratory DNA typing protocol.

B. POLYMERASE CHAIN REACTION

While not a DNA typing method in itself, polymerase chain reaction (PCR) is a technique that enables the amplification of subanalytical quantities of DNA to achieve a level that is conducive to DNA typing. The DNA probes used in conjunction with PCR are different from those used in RFLP testing. Probes used with PCR do not enable the individualization of DNA specimens. Moreover, these probes are currently unavailable commercially. Prices will become known when these probes become accessible to the community.

A DNA thermal cycler is needed to carry out the PCR technique. The cost of this instrument is approximately $10,000. Other equipment items are in common with the RFLP technique. Specialized reagent costs for the PCR technique will be about $10 per specimen.

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C. DNA SEQUENCING

A different method that can be used potentially to individualize body fluid specimens entails the direct determination of the sequence of molecules in DNA. While the application of this technique to forensic specimens is not as well-defined as the RFLP technique, it may be incorporated into the forensic laboratory within the next few years. The cost of a DNA sequencer approaches $100,000. Specialized reagent costs for this technique would be about $10 to $15 per specimen.

Table 1

EQUIPMENT ITEM	APPROXIMATE COST/ITEM
Autoclave (bench top)	$ 4,600
UV Spectrophotometer	12,000
UV Transilluminator	2,300
Electrophoresis power supply	2,500 (minimum of 2)
Electrophoresis tanks	400 (minimum of 4)
Microfuge (general use)	1,200
Microfuge (dedicated to isotopes)	1,200
Microliter pipettor (variable range)	250 (3 required)
Water bath	2,000 (2-3 required)
*Vacuum centrifuge	4,300
Ultra-low temperature freezer	6,300
*Environmental rotatory shaker	5,100
*Vacuum oven	1,000
Gel photography equipment	1,000
*X-ray film autoprocessor	7,100
Platform shaker	1,000
Benchtop radioisotope counter	2,400
OR	
Liquid scintillation counter	12,000
Radioactivity survey meter	250
Intensifying screens (pair)	300 (5 pair minimum)

Radiation Aspects of DNA Analysis

John P. Riley
Gunshot Residue and Metals Analysis Unit
FBI Laboratory
Washington, D. C.

Recent advances in DNA technology have provided the molecular tools which afford the forensic scientist the promise of the ultimate discriminating power, the ability to identify a biological specimen as having come from a single individual. The advanced DNA technology involves the extraction of the DNA from the biological specimen, cutting the DNA with restriction enzymes, electrophoretic separation of the cut fragments, transfer of the DNA to a membrane, the reacting of the transferred DNA with radioactive probes and the visualization of the location of the radioactive probes through the use of X-ray film. This developed X-ray film, called an auto-radiogram, provides the pattern which may allow individualization.

Since this process uses probes tagged with radioactive phosphorus 32, various aspects of radiation safety will have to be addressed when considering DNA analysis. Radioactive phosphorus 32 (^{32}P) comes under the purview of the Nuclear Regulatory Commission (NRC); therefore, it will be necessary to apply for an NRC Material License. At the time of application, the NRC will require one of the laboratory personnel to have training in radiation safety (from 1 day to a week's training, depending on the type of course attended) and some direct experience with beta-emission sources such as ^{32}P.

Another major consideration is a "hot laboratory" for processing samples with ^{32}P. A partial, but not all inclusive list of items needed in this laboratory includes a seamless hood, 1/2-inch plexiglass shielding, safety glasses, beta boxes for storage of ^{32}P, finger dosimeters, a hot sink and survey meters for the frequent monitoring of the laboratory for accidental spills and contamination. All of these items are available through commercial vendors. If your laboratory is already licensed to perform radioimmunoassay analysis, you may have most of this equipment, and you need only to amend your existing license to include ^{32}P.

Disposal of liquid waste containing ^{32}P must be performed according to Section 20.303 of Title 10, Code of Federal Regulations, part 20, which sets forth the pertinent NRC Rules and Regulations for disposal. Under most conditions, liquid ^{32}P waste can be immediately disposed of into the sanitary sewerage, whereas solid ^{32}P waste must be allowed to decay approximately 10 half-lives or approximately 5 months prior to disposal. Commercial waste firms are available for radioactive waste disposal, if necessary.

For laboratories with personnel unfamiliar with radioactive materials, the use of a consultant is recommended. Most large universities have radiation safety officers who could serve in this capacity by providing advice on how and where to purchase the ^{32}P and assist in obtaining training for personnel, acquiring the NRC materials license, outfitting the laboratory and establishing analytical procedure.

For further information, the following are commercial telephones numbers for the NRC Regional Offices:

Region I - King of Prussia, Pennsylvania
(215) 337-5000
Region II - Atlanta, Georgia
(404) 331-4503
Region III - Glen Ellyn, Illinois
(312) 790-5500
Region IV - Arlington, Texas
(817) 860-8100
Region V - Walnut Creek, California
(415) 943-3700

Training Considerations

A forensic scientist commencing study in DNA analysis will benefit from obtaining a theoretical background in the biological and chemical aspects of DNA formation, function, isolation and characterization.

To provide this type of background, the FBI Laboratory in conjunction with the Chemistry Department of the University of Virginia has designed a short course in molecular biology for FBI personnel. The course, entitled "DNA Characterization", will be taught by professors from the chemistry and biology departments of the University of Virginia. Successful completion of the 44 hours of lecture material will earn the student 3 semester hours of graduate credit. The course was designed to present a general overview into molecular biology topics and is not directed specifically toward forensic applications. Its purpose is to provide background material and supplement the theoretical and practical training in the forensic methodologies that are currently under development at the FBI. An outline of the topics to be covered are being listed to assist you in the potential development of similar courses with colleges and universities in your areas.

SHORT COURSE OUTLINE

Part I.

DNA structure, gene structure, chromosome structure, mitosis, meiosis, classical mapping techniques, human linkage maps, sex linkage.

a. DNA chemistry

Primary structure
Secondary structure
Tertiary structure
DNA denaturation and renaturation
Cot curves
Mismatch and renaturation

b. DNA organization in chromosomes

The nucleus
Nucleolus
Nucleus membrane
Nucleosomes and chromosome condensation

c. Extrachromosexual DNA

Mitochondria
Chloroplasts

d. Mitosis - the accurate copying of genetic information, somatic crossing over
e. Meiosis - independent assortment, crossing over and chiasmata

f. Mendelian genetics

Autosomal markers
Sex linkage
Family trees
Paternity questions

g. Mutations

Point mutations
Rearrangements
Internal crossing over
Natural selection
Polymorphisms (25% of all human loci)

Part II.

a. DNA replication

DNA polymerases
Exonucleases
Initiation, extension, termination
Ligase
DNA polymerization in vitro

b. Transcription

DNA - dependent RNA polymerases
Initiation, elongation, termination
Splicing

c. Translation

The adaptor hypothesis
Ribosomes
Protein synthesis
The genetic code; wobble
Protein polymorphisms

Part III.

DNA typing
DNA extraction and preservation stabilizing

a. Restriction mapping, partial digests, labeling techniques, DNA sequencing, autoradiography, blotting techniques, reprobing

b. Cloning technology

c. Simple and multi-copy polymorphisms
Human versus nonhuman DNA

d. Amplifying specific sequences of DNA with heat-stable DNA polymerase

e. Prospects for technical improvements

f. Linking classical genetic maps with DNA maps

DNA Bibliography

Harold A. Deadman
Forensic Science Research and Training Section
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FBI Academy
Quantico, Virginia

The following bibliography of papers and text chapters dealing with DNA analysis is divided into 15 topic areas. Some papers are historical in nature, however, most were recently published. These listings do not represent complete coverage of a topic area but contain selections that should provide sufficient background material necessary for the understanding of a particular subject. The purpose of this bibliography is to provide resource material that is directly related to both the theoretical and practical aspects of the forensic analysis of DNA.

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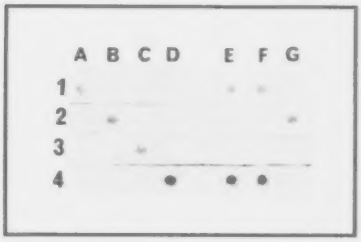

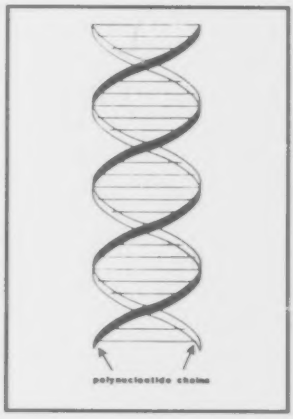

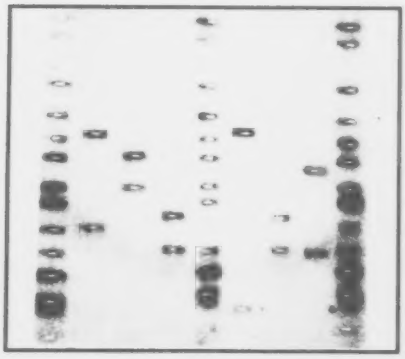

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The Crime Laboratory Digest is published quarterly by the FBI Laboratory in cooperation with the American Society of Crime Laboratory Directors (ASCLD). It is intended to serve as a rapid means of communication between crime laboratories, permitting information of interest and value to be disseminated among crime laboratory scientists.

Inclusion of an article in the Crime Laboratory Digest in no way represents an endorsement or recommendation of any part of that article by the Federal Government, the Department of Justice or the FBI. Contributing authors assume total responsibility for the contents and accuracy of their submission. Questions or requests concerning an article should be directed to the contributing agency.

All submissions are subject to editorial review in accordance with the editorial policy established by the FBI Laboratory and ASCLD. The editorial staff of the Crime Laboratory Digest reserves the right to edit all articles for style, grammer and punctuation. Comments and letters to the editor are encouraged and will be published when appropriate and as space permits. These should be forwarded to:

Crime Laboratory Digest - Editor
FSRTC, FBI Academy
Quantico, Virginia 22135

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ON THE COVER

The cover, prepared by the Special Projects Section of the FBI Laboratory, depicts dot blots of amplified DNA (top left), image analysis of RFLP patterns of a DNA autoradiogram (bottom left) and a DNA double stranded helix (right)

Message from the Assistant Director in Charge of the FBI Laboratory

In the January 1988 issue of the Crime Laboratory Digest, a review article appeared which discussed the principles of DNA analysis. The article also described the FBI Laboratory's research approach to DNA profiling. A supplemental issue of the Crime Laboratory Digest followed in April 1988 which provided information on the various consideration factors for implementation of DNA in the forensic laboratory. In June of 1988, a seminar on DNA technology was held at the FBI Laboratory's Forensic Science Research and Training Center (FSRTC), Quantico, Virginia. This seminar was attended by 100 scientists from private sector laboratories, academia and the forensic community who are actively involved in studying the issues surrounding the implementation of DNA analysis in the forensic laboratory. A summary of the technical presentations at this seminar was published in the July 1988 issue of the Crime Laboratory Digest. In June of 1989, a symposium on DNA analysis will be hosted by the FBI Laboratory at the FBI Academy. Over the past year, a tremendous effort in time and resources has been put forth by the FBI Laboratory to conduct the critically needed research in DNA analysis as it applies to forensic science, with the goal of implementing DNA analysis on casework by the end of 1988. In addition, there has also been a considerable effort to keep the forensic community informed of the progress made by the FBI to meet this goal.

As we conclude this year, I can report that we have begun the analysis of DNA on casework material. This entire issue of the Crime Laboratory Digest presents the summary of the work we have done during the past year on DNA research and casework implementation including a description of: 1) the research on extracting DNA; 2) the techniques we are using for DNA analysis; 3) a semiautomated analysis system for reading autoradiograms; 4) the results of the validation of the procedure; 5) considerations for quality control; and 6) the FBI Laboratory policy on examining DNA evidence. There is also a summary of the activities of a technical working group on DNA analysis methods which the FBI Laboratory has organized from members of the forensic community. This group will continue to meet over the next year to address such topics as quality control, standardization of methods and DNA database development.

The FBI Laboratory has established a collaborative research and training program for DNA analysis. This past fall six visiting scientists from state and local forensic laboratories completed a 4-month research program at the FSRTC. Additional visiting scientists are participating in this program, and so far 16 visiting scientists have participated in this program. In January 1989, the FBI Laboratory is offering the first of two classes to state and local crime laboratories on DNA analysis at the FSRTC. Each class is 4 weeks long and is a combination lecture/laboratory course which is accredited through the University of Virginia.

The thrust of the entire FBI's DNA program, from research to implementation to training, is to promote successful DNA analysis at the state and local levels. Initially, we expect that many cases will be submitted to the FBI Laboratory for DNA analysis, but with successful training of state and local crime laboratory personnel, DNA analysis will be performed locally.

ROGER T.CASTONGUAY

Editor's Column

We complete Volume 15 for the Crime Laboratory Digest with this issue. The editorial staff would like to take this opportunity to thank the following individuals for contributing their time and energies in reviewing articles submitted for publication in the Crime Laboratory Digest: Rod Collins, Ray Moore, Keith Monson, Dean Fetterolf, Robert Cravey, Irving Stone, John Murdock, Drew Richardson, Clarence Phillips and Peter DeForest. Without the efforts of these individuals, we would not be able to provide peer review on the diverse topics we publish.

The focus of this issue is the FBI Laboratory's success in bringing DNA analysis from a research effort to implementation on casework. The message from the Assistant Director in Charge of the FBI Laboratory highlights the efforts in bringing DNA analysis to casework.

The United States Department of the Interior's National Fish and Wildlife Forensic Laboratory has opened in Ashland, Oregon. This new 23,000 square foot laboratory is committed to providing a service to the 200 Special Agents from the Department of Interior's Division of Law Enforcement, to assisting all 50 state fish and game agencies and to helping the 97 signatory countries of the United Nations Convention on International Trade in Endangered Species. The director of the laboratory, Mr. Ken Goddard, can be reached by telephone on 503-482-4191, FAX: 503-482-4989), and the address is National Fish and Wildlife Forensics Laboratory, 1490 East Main Street, Ashland, Oregon 97520. The National Fish and Wildlife Forensic Laboratory will host the Spring 1989 meeting of the Northwest Association of Forensic Scientists. Details appear in the meeting announcement section.

EMPLOYMENT OPPORTUNITIES

Clerical positions: The United States Department of the Interior's National Fish and Wildlife Forensic Laboratory has openings for a variety of clerical positions in its new facility in Ashland, Oregon. For more information on the positions that are available, contact the National Fish and Wildlife Forensics Laboratory, 1490 East Main Street, Ashland, Oregon 97520 (telephone: 503-482-4191). United States citizenship is required. The United States Government is an Equal Opportunity Employer.

Criminalist I, II or III: The Houston Police Department Crime Laboratory has a position open for a criminalist at level I, II or III. Qualifications for the Criminalist I include a Bachelor's degree in chemistry, forensic science or a related science field with 40 hours of chemistry. Certain related scientific courses may be substituted for chemistry. Requirements for the Criminalist II include qualifications for the Criminalist I plus 2 years experience in a forensic laboratory. Requirements for the Criminalist III include qualifications for the Criminalist I plus 4 years of experience in a forensic laboratory. Duties consist of performing professional analytical laboratory work dealing with scientific crime detection. Salary will be determined by the candidate's qualifications. Send resumes, with salary history to: City of Houston, Personnel Department - AC/CRIM, 500 Jefferson, 15th Floor, Houston, Texas 77002. The city of Houston is an Equal Opportunity Employer.

Forensic Specialists: The recently opened United States Department of the Interior's National Fish and Wildlife Forensic Laboratory is seeking to fill a number of positions in a variety of forensic specialties in fish and wildlife enforcement. For information on current openings contact Mr. Ken Goddard, Director, National Fish and Wildlife Forensics Laboratory, 1490 East Main Street, Ashland, Oregon 97520 (telephone: 503-482-4191). United States citizenship is required. The United States Government is an Equal Opportunity Employer.

Senior Criminalist: The city of Mesa Police Department is seeking applicants for a senior criminalist position. Minimum qualifications include a Bachelor's degree in chemistry, criminalistics or a closely related field and 4 to 6 years experience as a criminalist. The individual that fills this position will specialize in areas of blood alcohol and drug screening in body fluids. Duties also include preparation of

reports and testifying in court as an expert witness. Salary range: $36,283 - $48,971 per year. An application may be obtained by mailing a resume to the City of Mesa Personnel Department, P. O. Box 1466, Mesa, Arizona 85211-1466 (telephone: 602-644-2365). The city of Mesa is an Equal Opportunity Employer.

Trace Evidence Analyst or Serologist: The Arkansas State Crime Laboratory is accepting applications for a position in either trace evidence analysis or serology. Requirements are a Bachelor's degree with a major in chemistry or biology plus relevant forensic laboratory experience. Skilled testimony as an expert witness and effective oral/written communication abilities are also required. Duties include analysis of hairs, fibers, gunshot residue, accelerants, paint, soil and glass. Salary will be determined by the candidate's qualifications. Applications should be submitted to Bill Cauthron, Executive Director, Arkansas State Crime Laboratory, #3 Natural Resources Drive, P. O. Box 5274, Little Rock, Arkansas 72215 (telephone: 501-227-5747). The State of Arkansas is an Equal Opportunity Employer.

PROCEEDINGS OF INTERNATIONAL SYMPOSIA PUBLISHED BY THE FBI LABORATORY

The following proceedings of international symposia hosted by the FBI Laboratory are currently available from the Government Printing Office:

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The following two symposia proceedings were previously available from the Government Printing Office but are no longer available from this source. These proceedings are now available from the National Technical Information Service:

The Proceedings of the International Symposium on the Analysis and Detection of Explosives, order number PB88-248562, price $38.95 plus $3.00 shipping and handling.

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A selected bibliography on polygraph countermeasures has been compiled by Dr. John A. Podlesny, Researcher, and Colleen Wade, Librarian, FBI Laboratory Division. Permission has been granted by the publishers to disseminate copies of the documents cited in the bibliography. This service is provided for all duly authorized crime laboratories. To obtain copies of the information, forward a written

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The FBI Laboratory Division has contracted with the Aspen Systems Corporation, an affiliate of the National Criminal Justice Reference Service, to index the back issues of the Association of Firearm and Toolmark Examiners Journal. The index to date ranges from 1969 through 1986 and includes 1,490 indexed articles. Each subsequent year of the journal will also be indexed. This index is available in printed and electronic formats.

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Technical Article

Recovery of DNA from Human Biological Specimens

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DNA preparations must meet several criteria prior to their introduction to the restriction fragment length polymorphism (RFLP) analysis. The DNA should be of high molecular weight, it should be free of impurities that might inhibit the catalytic activity of a restriction endonuclease (RE), and the quantity of DNA must be adequate for the analytical demands of the methodology. The source of the biological specimen from which the DNA will be recovered will determine the ease with which these criteria are met. A considerable research effort has been expended to optimize methods that facilitate the recovery of suitable DNA from the three broad specimen categories encountered in the forensic laboratory: liquid bloods, dried stains and vaginal swabs. The approaches developed for each category will be described separately.

LIQUID BLOOD SPECIMENS

Blood specimens from known sources should be collected in tubes containing ethylenediamine tetraacetic acid (EDTA) as the anticoagulant. In addition to serving as an anticoagulant, EDTA inhibits the activity of enzymes that act to degrade DNA. Blood collected in tubes without an anticoagulant yields DNA of both reduced quantity and quality, and its submission in this form for DNA typing should be discouraged.

DNA is recovered from liquid blood specimens after freezing and thawing, digestion with proteolytic enzyme/detergent, phenol: chloroform extraction and ethanol precipitation. Liquid blood specimens routinely yield at least 20 to 25 μg DNA/ml blood with an A260/280 of 1.6-1.8. No difficulties have been experienced in the digestion by REs of DNA prepared by our procedure.

The procedure for the recovery of DNA from liquid blood specimens has a different sequence of steps than the procedure used for dried stains. To avoid the inconvenience that these procedural differences introduce to the concurrent processing of liquid and dried specimens, dried stains are prepared from the liquid blood specimens immediately upon receipt. The processing of known specimens as dried stains insures that all items from a case submission will be treated in an identical manner.

DRIED BODY FLUID STAINS

Among the vagaries that can be active in reducing the yield of DNA from stains are: (1) dehydration of the stain and (2) penetration of the fluid into recesses of the surface onto which it has been deposited. The methods for recovery of DNA from dried stains have been evaluated on stains prepared with fixed volumes of blood, semen or saliva. These stains were prepared on an assortment of materials of varying penetrability and were exposed to environmental conditions known to abrogate the typeable characteristics of protein genetic markers.

Stains are routinely cut and extracted in a solution composed of detergent, reducing agent, EDTA and proteolytic enzyme for at least 16 hours at 56° C. The stain extracts are then treated once with phenol:chloroform, and the DNA is precipitated with ethanol. An improved yield of DNA could be demonstrated by lengthening the extraction time period for up to 4 days; however, there was some reduction in the quality of the DNA recovered after prolonged extraction time. The quality and quantity of DNA recovered from stains are determined in a test agarose gel containing quantitative standards of DNA.

This procedure has been used successfully to recover DNA patterns which are acceptable for typing from dried stains of blood, semen and saliva, as well as from follicular cells attached to anagen hairs.

VAGINAL SWABS

Evidence swabs used to collect semen from

the vaginal cavity will contain cellular elements representative of both the male and female. If the number of spermatozoa on the swab are low relative to the number of vaginal epithelial cells, most of the DNA recovered will be of female origin. If such a sample is diluted to avoid gel overloading, there is the risk that the sperm cell DNA will be diluted to a level that is below the limit of detection. Such a scenario can be avoided by recovering the epithelial cell DNA separately from the sperm cell DNA. We have adopted the procedure described by others and found it to be acceptable, with only a slight modification, for the processing of vaginal swabs. Briefly, swabs are initially extracted in buffered saline for 1 hour. A small volume of extract is removed for semen identification procedures. After the addition of detergent and proteolytic enzyme, the incubation is continued for at least another 16 hours. This extract will contain the DNA from the female's epithelial cells. The swab and pellet are extracted again in the presence of a reducing agent that lyses sperm heads and releases male DNA. Using swabs prepared in the laboratory and processed by this scheme, we have successfully obtained RFLP patterns from vaginal swabs containing as little as 1 μl of semen.

CONCLUSION

Methods have been described for the recovery of DNA from the several categories of specimens encountered in the forensic laboratory. While these methods were refined using laboratory-prepared samples, they have been employed successfully for the examination of nonprobative case evidence stains.

Technical Article

The RFLP Technique

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Molecular biology techniques afford the forensic serologist the potential to identify a body fluid/tissue donor to the exclusion of all other individuals via DNA typing. One analytical approach that can achieve this goal is the restriction fragment length polymorphism (RFLP) technology. The Forensic Science Research Unit of the FBI Laboratory has made a major effort in developing a RFL methodology and validating the application of RFLP analysis to forensic biological specimens. The product of that research has come to fruition, and this summary provides the forensic community the salient features of the methodology.

The points to stress are that this RFLP technique is extremely sensitive, reproducible, reliable, simple and relatively inexpensive. To achieve these qualities, the following areas were addressed: choice of restriction enzyme, capillary transfer approach, choice of membrane, prehybridization and hybridization conditions, and selection of probes.

Restriction endonucleases (RE)s are enzymes derived from bacteria that catalyze the cleavage of double-stranded DNA. The class II REs will cleave DNA only if a specific sequence of bases is present. Since the samples encountered at crimes scenes are not of a pristine quality, it was deemed necessary to select a RE that is robust. Further, it was necessary to choose an appropriate enzyme first and then determine what probes would be compatible with that enzyme, not the converse. Hae III is the restriction enzyme of choice. It recognizes the four nucleotide sequence GGCC and forms a blunt end cut between the G and C. Our investigations and the literature (Blakesley et al. 1977) demonstrate that Hae III is an extremely hardy enzyme. Under less than optimum conditions, such as temperatures as high as 70° C, ionic strengths varying from 25mM to 150mM and a pH range of 6 to 9, Hae III cuts at its restriction site with no adverse effects. Further, Hae III, recognizing a four base sequence, will cleave the DNA fragments into smaller pieces than REs that recognize larger sequence sites. Due to the nature of electrophoresis, the smaller the generated fragments, the more likely subtle genetic differences (manifested as fragment size variants) can be resolved. In fact, a comparison of the restriction enzymes Pst I, Alu I, Hinf I and Hae III demonstrated that the best resolved patterns for the probe system YNH24 (D2S44) were created using Hae III. Finally, the methylation scenario of higher eukaryotes has no impact on the ability of Hae III to recognize and cleave its particular restriction site. Thus, DNA derived from different body tissues of an individual will not likely produce different fragment patterns.

After electrophoresis of the digested DNA, the array of DNA fragments in the gel is transferred to a nylon membrane by Southern blotting (Southern 1975). Southern blotting transfers by capillary action, the DNA out of the gel onto a nylon membrane where the fragments readily bind. The transfer solution is 0.4 M sodium hydroxide. Sodium hydroxide denatures double-stranded DNA so single-stranded target site DNA is presented for subsequent probing. By transferring in sodium hydroxide, a single-stranded nature for DNA is maintained at the time of membrane fixation. This approach eliminates the need for a neutralization step prior to blotting. Further, it has been demonstrated that transferring in a low ionic strength, alkali environment is more efficient for binding DNA to positively charged nylon membranes (Reed and Mann 1985).

To effectively bind small fragments of DNA, a positively-charged nylon membrane is selected. The DNA can be fixed to the membrane after blotting by air drying, heat baking or exposure to UV light.

It once was dogma that the membrane was washed overnight in a prehybridization solution to block unoccupied sites. This step has been eliminated. The membrane simply is placed in the hybridization solution, and subsequently, boiled probe is added. Many of the components routinely used for hybridization have been eliminated. The basic hybridization solution contains only 7% SDS, 10% PEG (MW 8000) and phosphate buffer.

There have been three major effects obtained using this simplified hybridization solution. They are an increased sensitivity of detection, reduced membrane background and easier troubleshooting of defective reagents.

One of the most important aspects of an RFLP methodology for sensitivity of detection and statistical evaluation is the probe systems applied for analysis. Observations by Wyman and White (1980) revealed that between restriction sites and within noncoding regions of human genomic DNA were multiple repeats of nucleotide sequences. Moreover, the number of repeats of these core sequences was shown to be highly variable within the human population and to be inherited in a Mendelian fashion. Probes that recognize these variable number tandem repeats confined to a single locus provide a high degree of sensitivity and cross-react only with higher primates. Some variable number tandem repeat sequences have been reported to exhibit a high degree of polymorphism such that analysis of a sample with three to five probe systems can provide individualization. The two or one band patterns generated with those probes present simple profiles so standard statistical approaches can be applied, and interpretation of mixed body fluids is more readily performed. The following probes have been evaluated and found to be effective for characterization of body fluid stains: YNH24, CMM101, 3'HVR, MS1, V1, L255 and L365. As long as pattern matching of questioned and known samples is performed, all these probes should be quite useful. Probes that detect high mutation rate sites such as MS1 should be avoided for paternity testing.

The purpose of this summary was to provide a brief report on certain aspects of RFLP technique developed by the Forensic Science Research Unit of the FBI Laboratory. The method is simplistic, sensitive and reliable. More explicit information on the technique will be provided in the appropriate journals.

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Technical Article

The Use of DNA Amplification in the Analysis of Forensic Evidence

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The detection of DNA polymorphisms in forensic samples by analysis of restriction fragment length polymorphisms (RFLP) is a powerful technique for determining the identity of these samples (Baechtel 1988; Budowle 1988; Budowle *et al.* 1988). This technique requires at least 100 ng of relatively undegraded DNA. However, some forensic samples do not contain this much DNA. Some examples would be very small blood or semen stains, stains that contain degraded DNA due to the environmental conditions that the stains were subjected to, and single hairs. For these applications, the technique of polymerase chain reaction, or PCR, combined with an allele detection technique such as hybridization of dot blots, is proving to be a useful tool for analysis of DNA polymorphisms.

The polymerase chain reaction (Saiki *et al.* 1985), using the thermostable enzyme *Taq* polymerase (Saiki *et al.* 1988), is a technique which amplifies DNA by a series of heat denaturations, primer annealing, and primer extension. During heat denaturation, the double-stranded DNA molecules are converted to single-stranded molecules. The reaction temperature is then lowered to allow for annealing of the primers, which delineate the region to be amplified, to the single-stranded DNA. The temperature is raised to the optimum temperature of the *Taq* polymerase, 72° C, and complementary DNA strands are synthesized, using the annealed primers as initiation sites for the polymerase. This series of denaturation, annealing, and extension is repeated for many cycles, perhaps 30 to 35 cycles, using the DNA Thermal Cycler (Perkin-Elmer-Cetus, Norwalk, CT), allowing many copies of the original DNA sequence of interest to be synthesized.

The current research effort in the FBI Laboratory using PCR involves amplifying a region of one of the major histocompatibility complex genes, DQ-alpha. This class II antigen gene, like other HLA genes, is very polymorphic. There are four nominal alleles, 1, 2, 3 and 4, and the 1 allele can further be subtyped into 3 alleles, 1.1, 1.2, and 1.3. Therefore, there are 21 possible genotypes from these 6 alleles. The population frequencies for the different genotypes range from 0.0012 to 0.12. The variations which determine these alleles are located in a 242 bp region of DQ-alpha. Primers which flank this region are used in the amplification, and allele-specific oligonucleotide (ASO) probes have been developed to detect each of the DQ-alpha alleles (Higuchi *et al.* 1988).

AMPLIFICATION OF DNA EXTRACTED FROM DIFFERENT TYPES OF SAMPLES

Liquid Blood

DNA was extracted from frozen liquid blood according to the procedure used in the FBI DNA laboratory. The DNA was then amplified using PCR as described by Saiki *et al.* (1988). The temperatures used during the cycling were 93° C for denaturation, 55° C for annealing, and 72° C for extension, with cycle lengths of 1, 2 and 3 minutes, respectively. Starting with 200, 20, 1, 0.1 and 0.01 ng of genomic DNA from liquid blood, the 242 bp region of the DQ-alpha gene could be amplified. Every sample of DNA from frozen liquid blood that has been subjected to PCR has amplified. Figure 1 shows the amplified 242 bp fragment of the DQ-alpha gene from three different liquid blood DNAs.

Figure 1. PCR amplifications of liquid blood DNA. The arrowhead indicates the position of the 242 bp fragment from the DQ-alpha gene. Lanes 1, 2, and 3 represent different DNA samples. Lane M is a DNA size marker.

Bloodstains

DNA was extracted from bloodstains on cotton sheeting using the protocol described by Baechtel (1988). The stains varied in age from 1 to 9 months. One was frozen at -70° C before it dried, others were dried and stored at -70° C or room temperature. The quality of the extracted DNA was assessed by observing the appearance of the DNA on ethidium bromide-stained agarose gels. When subjected to PCR, some, but not all, of the samples amplified. In fact, the genomic DNAs which were least degraded and present in the highest concentration did not initially amplify. Additional ethanol precipitation of the DNA or spin dialysis of the DNA using Centricon concentrators (Amicon, Danvers, MA) did not purify some of the samples enough to be amplified. However, these samples could be amplified if tenfold less DNA was used. This result indicates that DNA from bloodstains contains a contaminant which inhibits amplification and is resistant to removal by the standard extraction procedure. This is supported by results of an amplification experiment using the DNA which initially amplified well. If tenfold more DNA was used, less amplification was actually seen. Higuchi (personal communication) has suggested that this contaminant may be heme. However, this contaminant does not appear to be present in DNA from liquid blood.

Semen Stains

DNA extracted from 1 μl semen stains, using the standard protocol for semen stain extraction (Baechtel 1988), can be readily amplified.

Hairs

DNA was extracted from roots of single telogen hairs, roots of forcibly removed hairs (containing sheath material) and single hair shafts, according to the procedure described by Blake *et al.* (1988). The DQ-alpha gene can be amplified from the DNA from single telogen hair roots and single roots of forcibly removed hairs, but not from individual hair shafts.

Vaginal Swabs Containing Semen

DNA was extracted from vaginal swabs to which semen had been added. The differential extraction procedure described by Baechtel (1988) was used. Each of the two fractions containing vaginal cell DNA and the fraction containing sperm DNA were subjected to PCR, and the DNA contained in each of them amplified.

ANALYSIS OF DQ-ALPHA PHENOTYPES BY DOT BLOTTING

Two formats of the dot blot procedure described by Higuchi *et al.* (1988) and Saiki and Erlich (1988) are being explored in the FBI Laboratory. The principles of each, as well as the advantages and disadvantages of each method, will be described in turn.

Immobilized Amplified DNA

In the procedure described by Higuchi *et al.* (1988), the amplified DQ-alpha DNA is applied to a nylon membrane using a dot-blot apparatus attached to a vacuum. For each sample, replicate dots are made for each ASO probe. Samples are aligned so that replicate dots are arranged vertically and different samples are arranged horizontally. The membrane can then be cut in horizontal strips, and each strip can then hybridized with a different ASO probe. Each ASO probe is conjugated with horseradish peroxidase (HRP). Following hybridization, the HRP, and hence a particular allele, can be detected using a substrate for peroxidase which is converted from a colorless form to a blue precipitate. Figure 2 shows DQ-alpha typing of amplified DNA from each of the two vaginal DNA fractions and the semen DNA fraction from a vaginal swab to which semen had been added. Also, the DQ-alpha typing of amplified DNA from four known homozygous cells lines is shown.

Although this procedure is not sensitive enough to detect DQ-alpha genes in small, unamplified samples, amplified DNA from any source can be detected using this method. The number of hybridizations is determined by

the number of probes, because many different samples can be affixed to a single membrane and hybridized with a single probe.

A major drawback of this method involves the application of multiple samples to the dot blot apparatus. Carelessness could lead to applying two samples to a single dot and/or skipping dots. Additionally, care must be taken so that the replicate applications for each sample are kept in the same order so correct interpretation of the DQ-alpha genotype can be made. Currently, the probe for allele 1 detects a weak signal and also gives some background signal. Modified probes are being developed which may alleviate these problems (Higuchi personal communication).

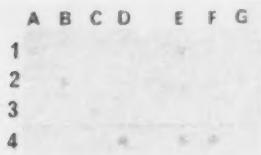

Figure 2. Columns A-D: DQ-alpha typing of homozygous cell line DNA. Amplified DNA from four homozygous cell lines was applied to a nylon membrane in a pattern of four dots per sample. Column A is DNA from a cell line homozygous for allele 1, B is homozygous for allele 2, C is homozygous for allele 3, and D is homozygous for allele 4. Columns E-G: DQ-alpha typing of vaginal swab DNA DNA was differentially extracted from a vaginal swab to which semen had been added. DNA from two vaginal fractions (E and F) and the semen fraction (G) was amplified, and a portion of the amplified DNA was applied to a nylon membrane in a pattern of four dots per sample. The membrane was cut into four horizontal strips and each strip was hybridized with an ASO probe, indicated by 1-4. The vaginal DNA has a DQ-alpha genotype of 1, 4, and the DNA from semen is homozygous for allele 2.

Immobilized ASO Probes

An alternate method of DQ-alpha gene detection entails using strips of nylon membranes, each of which has each ASO probe affixed to it in a horizontal array (Saiki and Erlich 1988). The PCR reactions must be done with biotinylated primers. The amplified DNA is then added to the hybridization solution, which contains streptavidin conjugated to HRP. During hybridization, the streptavidin binds to the amplified DNA molecules, each of which contains biotinylated primers, and the amplified DQ-alpha DNA hybridizes to the ASO probes it matches. Hybridization is detected by a color development assay using a substrate for peroxidase. Figure 3 shows the pattern of the DQ-alpha probes on the nylon membrane and the expected hybridization pattern for an individual of the genotype 1, 3, with a 1 subtype of 1.1.

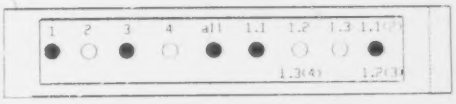

Figure 3. Schematic for Immobilized ASO Probe method of DQ-alpha typing. The filter has 9 probes affixed to it in a horizontal array. The first four probes are for alleles 1-4. The next probe, "all", hybridizes if any DQ-alpha DNA amplifies at all. The next four probes detect one or more alleles and are used for subtyping for allele 1. Two of the probes, as indicated in the schematic, detect more than one allele, but by analyzing the pattern of hybridized dots the allele 1 subtype can be determined. The pattern shown is for the genotype 1, 3, with the allele 1 subtype 1.1.

Using this method, the number of hybridizations necessary is equal to the number of amplified samples. For a small number of samples, this is an efficient procedure. An individual's DQ-alpha type can be read from a single membrane strip, instead of from several strips as required by the immobilized amplified DNA method. The possibility for errors caused by application of many samples to a dot blot apparatus is eliminated. However, for a large number of samples, it becomes difficult to

manipulate the large number of separate hybridizations, which are done in individual heat-sealable plastic bags. It may be possible to buy the membranes already inserted into bags, which would alleviate some of the manipulation problems. The problem with the weak signal for allele 1 is also present with this method, but, again, the development of a new probe for this allele should solve this problem.

CONSIDERATIONS FOR SAMPLE CONTAMINATION IN THE CRIME LABORATORY

The use of PCR combined with the dot blot method for typing DNA obtained from forensic samples provides a very sensitive analysis procedure. However, the sensitivity of this test also means that contaminating DNA samples can also be detected, and great care must be taken to avoid this. The most serious contamination problem arises from the contamination of genomic DNA or components of the amplification reaction with amplified DNA. The number of copies of an amplified gene can be calculated by the formula $(x)(2^n)$, where x is the original number of copies before amplification and n is the number of cycles. If x is equal to 40, or the number of copies in 20 cells (140 pg of genomic DNA, assuming 7 pg DNA per cell), and the cycle number is 30, than at the end of cycles of PCR there will be 4.3×10^{10} copies of the gene. If the PCR was done in 100 μl, then 0.1 μl will contain 4.3×10^7 copies. This 0.1 μl, which could be transferred to another sample via aerosol arising from opening a tube, from a gloved hand, or from a pipet, among other possibilities, would introduce far more copies of the DQ-alpha gene than would be present in most forensic samples, and, after amplification of the contaminated sample, only the DQ-alpha type of the contaminant would be determined. Contamination can also arise through introduction of genomic DNA from one sample into a tube with genomic DNA from another sample. If both samples contained approximately equal concentrations of DNA, a small amount of sample transfer would not be detected. However, if, for example, a DNA preparation from liquid blood was prepared alongside a DNA preparation from a hair, a small volume of transfer from the liquid blood sample to the hair sample would introduce significant contamination as measured by gene copy number. After amplification and typing of the contaminated hair DNA sample, it is possible that the types of both DNA samples will be visible.

The contamination problems described above can be detected and avoided by the use of proper control samples and care in the workplace. The most effective way to avoid contamination by amplified DNA is to separate the sample preparation area from the area in which amplified DNA is handled. No reagents or pipets which come into contact with amplified DNA should ever be used with or near pre-amplified samples. Samples of known DQ-alpha type and samples with no DNA (but containing all of the components of the PCR reaction) should be run in parallel with every amplification and dot blot. These controls will detect any contamination of reagents. Cross-contamination of genomic DNA samples can be minimized by only preparing samples of similar DNA concentrations at a particular time.

CONCLUSIONS

The polymerase chain reaction technique, combined with determination of DQ-alpha type by dot blot hybridization, is a procedure which will be very useful in the analysis of forensic samples, especially for samples which are not amenable to RFLP analysis. It will also prove useful as a tool to analyze DNA samples before RFLP analysis is done, because it offers more discrimination than other serological techniques currently being used and only takes 1 to 2 days to perform, whereas RFLP analysis can take a few weeks. Some further studies with this technique are necessary in the FBI Laboratory before the technique can be applied to casework, and are currently being undertaken. Some population frequency data are currently available on the DQ-alpha alleles, but a larger population study needs to be done. Studies of the effects of various environmental conditions and hazards on amplification and typing of DNA, similar to the studies of these effects of RFLP analysis done by Adams (1988), are also being undertaken.

This technique does not offer the degree of individual discrimination that RFLP analysis can; however, amplification of other DNA can

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offer more discrimination. One area which the FBI Laboratory is investigating is the amplification of mitochondrial DNA. The mitochondrial genome, which is about 16,500 bases, is present in hundreds of copies per cell. The mitochondrial genome has a region of about a 1,000 bp, called the D-loop, which is quite variable among individuals. We have amplified different parts of the D-loop and are sequencing the amplified DNA directly by the method of Mitchell and Merril (1988) in order to determine which part of the D-loop offers the most potential for discrimination. Because of the many copies of mitochondrial DNA per cell, analysis of mitochondrial DNA can be a more sensitive technique than analysis of a single copy gene such as DQ-alpha. Another area of research being pursued by the FBI Laboratory is the amplification of regions containing length polymorphisms. After amplification of such regions, analysis can be performed simply by observing the length differences on ethidium-bromide stained agarose gels. Combination of more than one of the above-mentioned systems will potentially permit a great deal of individual discrimination.

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Technical Article

Semiautomated Analysis of DNA Autoradiograms

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The necessity to rapidly and objectively analyze hundreds or thousands of forensic DNA autoradiograms requires that computer assisted methods of analysis and data management be developed. Each of the conventional genetic markers used in forensic serology generally exists in only 5 or 6 phenotypes, so that assignment of types is relatively straightforward. Analysis of Restriction Fragment Length Polymorphisms (RFLP)s is more difficult due to the large number of possible alleles.

A highly polymorphic RFLP probe may exist in nearly 100 alleles in human populations (YNH24, for example, has 71 known alleles). When 14 cm gels are used we may then need to resolve restriction fragments separated by about 1 mm. The width of bands on the autoradiogram makes accurate manual location of bands difficult at best.

A second major limitation of manual reading of autoradiograms is a consequence of non-uniform mobility across the width of the gel. Any local differences in field strength due to variations in ionic strength or gel thickness will cause species of the same size and molecular weight to migrate slightly different distances in different regions of the gel. Several control lanes containing size standards are included on each gel to monitor this phenomenon. Their presence permits match/nonmatch judgments to be made between nearby lanes but visual comparisons are inadequate when alleles of nearly the same size occur in widely separated lanes, or if inter-gel comparisons are necessary.

Although conventional serological markers are represented by a relatively small number of phenotypes, an RFLP analysis can reveal a relatively large number of genotypes. A quantitative measure of RFLP allele size is necessary to establish the population statistics for frequency of occurrence of each allele, which will in turn be used to estimate the probability that two individuals could have the same DNA profile. Quantitative results could also be used to establish a data base of convicted offenders which could be searched along with other criminal records.

FBI LABORATORY SYSTEM FOR SEMI-AUTOMATED ANALYSIS OF RFLPs

The necessity for a computer-assisted method for analysis of RFLPs was recognized early in the FBI Laboratory's program to implement DNA technology. Since no commercial product existed, the Forensic Science Research Unit was tasked with development of a system to serve research and caseworking needs of the FBI Laboratory and the larger law enforcement community.

The sequence of operations performed by the system developed at the FBI Laboratory is summarized in Table 1. Detailed explanations of each step will be provided in a later article; only an overview of each step will be presented here.

Generation of a digital version of the autoradiogram is a prerequisite to any type of computerized image analysis. At the FBI Laboratory we use an instrumentation-grade monochrome video camera to image the autoradiogram on a uniformly-illuminated light box. The video image is digitized at a geometric resolution of 480 lines by 512 columns and a gray scale resolution of 256 levels.

Routines for automatic location of lane boundaries, production of density profiles in lanes, and detection of band positions were originally developed at the National Institutes of Health (Pun *et al.* 1985) and extensively modified at the Forensic Science Research Unit. Automatic estimation of linear lane boundaries, with the option of subsequent manual override, is derived by statistical processing of the column-by-column standard deviation signal. Integrated intensity profiles for each lane are obtained by summing line scans for every column included by the lane boundaries. Bands are located by intensity ranking of local extrema in the density profile.

Geometric correction for nonuniform migration across the width of the gel is

calculated by assuming a linear variation in mobility between lanes containing size standards. The FBI Laboratory runs standards every 3 or 4 lanes across the gel to ensure that mobility variations are accurately modeled.

Lengths of restriction fragments (in base pairs) are calculated for bands in the specimen using the previously derived geometric correction function in conjunction with one or more functions which relate mobility to fragment length. Preliminary results indicate that a simple model which assumes that mobility varies as a function of the logarithm of molecular weight is quite accurate for quantification of the relatively short fragments produced by our restriction enzyme system, although we are also evaluating the more complex power law relationship proposed by Schaffer and Sederoff (1981).

Two distinct data bases are under consideration for management of data resulting from image analysis of DNA autoradiograms. First, a data base of allele frequency data for various racial groups and for each of the probes we are using is being generated by RFLP analysis of liquid blood samples. These data will be used to estimate the probability that one individual's DNA profile could be the same as another's. Second, we are evaluating the generation, maintenance, and dissemination of a data base of DNA profiles of known offenders, to which a suspect's profile could be compared. This type of data base is made possible because image analysis of RFLP patterns generates a few highly individualizing numerical results which could in principle be searched in the same way as a fingerprint classification.

IMPLEMENTATION

A prototype DNA analysis system was developed on a minicomputer and a general-purpose image processing system. A second prototype has been completed which runs efficiently on a personal computer (IBM PC/AT or compatible). The latter system was designed to be within the means of nearly any forensic laboratory (Table 2). Many well-equipped laboratories will already own all the equipment necessary to begin to analyze autoradiograms, with the exception of the frame buffer board. After appropriate testing of the system by selected laboratories, the software will be made available to all.

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Table 1

SEMIAUTOMATED ANALYSIS OF DNA AUTORADIOGRAMS

1. Digitize autoradiograph
2. Locate lane boundaries
3. Produce integrated intensity profiles
4. Locate bands from intensity peaks
5. Do geometric correction
6. Calculate molecular weights

Table 2

DNA IMAGE ANALYSIS EQUIPMENT

Item	Unit Cost
IBM PC/AT	$ 4,000
Camera stand	700
Video camera	2,000
Frame buffer	2,000
19" RGB monitor	1,500
Total	$ 11,200

Technical Article

Validation of the FBI Procedure for DNA Analysis: A Summary

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The technique of deoxyribonucleic acid (DNA) analysis by restriction fragment length polymorphism (RFLP) as described by Budowle (1988) requires the presence of relatively high molecular weight (HMW) DNA (10-20 kilobases) for successful typing. Because forensic evidence is often subjected to a variety of external influences prior to examination by crime laboratory personnel, an understanding of the impact of environmental insults or adventitious substances on DNA found in body fluids is paramount to interpretation of actual casework results. Experiments were designed to determine the effects of external influences on the quantity, quality and recovery of DNA in laboratory prepared body fluid stains. This paper is a brief description of results concerning the effects of the environment (sunlight and temperature), substrate and time on the stability of DNA derived from laboratory prepared body fluid stains. Additionally, stains made from body fluids mixed with adventitious substances were analyzed to simulate commonly encountered biological and chemical contaminants found in association with evidentiary items. Because all possible environmental, biological and chemical insults cannot be addressed experimentally, the final process of validation was to analyze, by the RFLP technique, the remnants of actual adjudicated case stains that had previously been submitted to the FBI Laboratory for traditional serological examination.

ENVIRONMENTAL RESULTS

Bloodstains of known origin maintained at ambient outdoor temperatures during March through May 1988, were sampled at biweekly intervals for 10 weeks to determine the effect of sunlight on DNA. The stains were divided into two equal portions with one set maintained in the dark and the other set exposed to the diurnal cycle of sunlight and darkness. Both sets of stains were exposed to identical temperatures which averaged approximately 21° C for the daily high temperature during the 10-week period. After 8 weeks of exposure to sunlight, DNA was degraded to the point where no RFLP banding pattern could be observed via autoradiography. However, DNA of sufficient HMW was recovered from stains maintained in the dark for the entire 10 week sample period. These stains as well as stains exposed to sunlight from 2 to 6 weeks, produced RFLP banding patterns identical to controlled, untreated bloodstains from the same individual.

A similar experiment was performed during July 1988, where the average daily high temperature was 41° C and the average daily low temperature was 23° C. The only difference in this study compared with the previous one was that bloodstained samples were harvested daily for a period of 12 days. Stains maintained in the sunlight for 10 days resulted in degraded DNA, while samples stored at the same temperature in the dark produced acceptable RFLP banding patterns to the conclusion of the experiment (Figure 1).

Figure 1. Autoradiogram representing results of DNA analysis of bloodstains from the same donor exposed to 12 days of sunlight (12 L) and 11 and 12 days in the dark (11 D and 12 D, respectively). The four bands in the first lane are size markers. The origin is at the top.

In another experiment, blood from known individuals was prepared as stains on a variety of porous and nonporous substrates which included cotton, nylon, denim, glass, wood and aluminum. These samples were divided into four replicate sets, and each set was subjected to one of four different temperatures (4° C, 22° C and 37° C and ambient outdoor). The daily high temperature during the sample period for the samples maintained outdoors ranged from 23° C to 41° C. Identical samples from each set were harvested after exposure for 2 days, 1, 3 and 5 months. With only minor exceptions, all samples, on all substrates, at all temperatures, produced RFLP banding pattern results consistent with untreated liquid blood samples of the same origin. Those stains subjected to even the most adverse environmental condition (5 months at 37° C) produced DNA patterns acceptable for typing. However, DNA is not stable at these conditions indefinitely as only low molecular weight (less than 100 bp) DNA could be detected from prepared bloodstained material maintained at 37° C for 4 years. No RFLP banding pattern results were obtained from these latter samples.

Contamination Results: Chemical

A chemical contamination study was designed to determine the influence of chemical substances in association with bloodstained material on the recovery of DNA and subsequent analysis by RFLP. The chemicals used in this study were representative of a variety of commonly encountered domestic substances which included unleaded gasoline, motor oil, detergent, acid, base, salt, bleach and soil. Blood from known individuals was prepared as stains on cotton sheeting already contaminated with one of these chemicals, with the exception of soil. In this case, blood was mixed directly with soil. The HMW DNA was recovered from all contaminated bloodstained samples analyzed 5 days later except those mixed with soil. The DNA in these later samples was probably degraded by enzymes present in the soil and subsequently produced no results following autoradiography. This theory is supported by the recovery of DNA from soil microorganisms isolated from soil samples not mixed with blood. No RFLP results were obtained for these samples either. All other samples where blood and a contaminant were present produced RFLP banding patterns consistent with the uncontaminated bloodstains from the same origin.

Contamination Results: Bacteria

Bacterial and/or yeast contamination can be encountered on evidentiary material. Two microorganisms used in this study are commonly found in the vaginal vault and are likely to be present on items of evidence following rape. These included *Staphylococcus epidermidis*, and *Candida valida* (yeast). Additionally, the ubiquitous *Escherichia coli* and *Bacillus subtilis* were used to determine the effects of these microorganisms on DNA found in blood and semen. Mixtures of each of the four microbes with either blood or semen were made, and stains were prepared from each mixture. Controls of uncontaminated blood and semen plus controlled samples of the cultured microorganisms were also analyzed. All analyses were performed 5 days after the stain preparation, and all results of the mixed stains showed RFLP banding patterns consistent with uncontaminated body fluid samples. DNA was also isolated from the microorganism controls (no blood or semen present), but no RFLP results were produced from DNA derived from these samples.

Mixed Body Fluids

The next study addressed whether or not body fluids mixed from different sources can be detected by RFLP analysis. An array of mixed body fluid stains were prepared from different donors. Body fluids analyzed consisted of semen, blood, urine, saliva and vaginal secretions. Each stain consisted of two body fluids from separate donors in all possible combinations. RFLP banding patterns were obtained on all samples containing vaginal secretions, semen and blood (except when only 1 μl of blood was used in the mixture) and in some cases, saliva. However, no RFLP results were obtained from urine. Mixing of body fluids did not affect the RFLP band positions compared with the controlled samples from the same donors.

Nonhuman Blood Samples

Blood samples obtained from nonhuman sources were analyzed to determine if RFLP banding pattern results could be obtained using the protocol for human body fluids. Only blood from primates produced results similar to those expected for humans, while all other DNA isolated from domestic and wild game animal bloods did not hybridize with the particular single locus probes utilized.

Evidentiary Stained Material

Bloodstained evidence (429 stains) representing the remnants from 122 different adjudicated cases submitted to the FBI Laboratory was analyzed. These samples were not subjected to any laboratory manipulation (other than freezing), but instead represented stains with unknown contaminants and unknown environmental history. These samples were 1 to 2 years old and had been frozen since their submission to the FBI Laboratory. Interpretable RFLP banding patterns were obtained in approximately 60% of the stains analyzed. No results were obtained from the remaining stains due primarily to sample degradation which was consistent with preliminary tests which provided evidence of DNA of low molecular weight. Undoubtedly, the lack of results for a portion of these latter stains was a function of not only the age of the stains and other unknown variables, but also previous serological examinations which likely consumed the most desirable portion of the stain.

CONCLUSION

In conclusion, these validation studies clearly indicate that of the more than 2,000 samples analyzed for DNA by the RFLP technique, the results were either consistent with known samples (when available) or no results were obtained due to predictable influences on the integrity of HMW DNA. Samples that were influenced by external insults at no time produced uninterpretable or questionable results that differed from controlled samples. The design of these experiments as described was to not only determine the validity of DNA analysis, but to also determine the reliability and reproducibility of this technique. These results strongly support the FBI Laboratory protocol for DNA analysis by the RFLP technique as valid, reliable and reproducible.

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Feature Article

Quality Control in DNA Typing: A Proposed Protocol

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With the introduction of deoxyribonucleic acid (DNA) typing technology to the forensic laboratory, the forensic examiner now has the potential to individualize various body fluids and tissues. This technology makes use of complex molecular biology procedures to detect polymorphisms that are expressed within the DNA itself.

As technology becomes more complex, it is essential that the quality of the analytical data be monitored to ensure that the accuracy and related performance characteristics of the procedure are maintained. Further, the nature, quantity and condition of forensic samples require appropriate standards and controls to ensure reliable and reproducible interpretation.

One way to provide reliable evidence that the data generated by an analytical procedure is scientifically sound is through the implementation of detailed, but flexible, quality assurance (QA) and quality control (QC) programs.

This paper presents proposed minimum requirements for a QA/QC program as applied to the DNA analysis of biological stains in criminal cases. Although often used interchangeably, QA and QC refer to specific quality functions (Whitehead and Woodford 1981; Kilshaw 1986). The function of QA is to provide evidence that the QC function is being performed adequately. This is accomplished through the use of external QA requirements that are administered by a group outside of the DNA Analysis Unit (Kilshaw 1987a), such as analysis of blind and open proficiency samples and audits. The QC functions are internal activities initiated by the DNA Analysis Unit (Kilshaw 1987b), which ensure that the quality of the product (DNA typing) is maintained and will meet and satisfy specified criteria.

Although the application of formal QA/QC programs to forensic laboratories is not widespread and little information has appeared in the forensic science literature (Brunelle *et al.* 1982; Pereira 1985), a great deal has been written on the application of QA/QC programs to clinical and federally operated laboratories (Kenney 1987; Kidd 1987; Taylor 1987; Hay 1988; Alwan and Bissell 1988; Bussolini *et al.* 1988).

QUALITY CONTROL FOR DNA TYPING

The QA/QC program proposed for DNA typing of forensic evidence is outlined in Table 1. This program was designed following a systematic study of the DNA typing process and covers all aspects of the laboratory process. The purpose of the program is to provide the necessary documentation to assure the laboratory managers and examiners that the process is operating within the established performance criteria.

QUALITY CONTROL MANUAL

The specific details of the QA/QC program and methods of analysis for DNA typing are described in clear and complete language in a QC manual. The manual is organized into sections as outlined in Table 1. Once it is approved, this manual provides guidelines for the standard operating procedure (SOP) for DNA typing, as well as guidelines for the QA/QC program. All changes and updates to the QC manual and to the SOP should be approved by the FBI Laboratory.

PLANNING AND ORGANIZATION

This section describes the scope, policy and objectives of the QA/QC program. The scope and objective(s) should explain what the program intends to provide (Ruzicka 1962;

ASQC 1968). The quality policy would outline the administrative responsibility for the QA/QC program for DNA typing.

QUALIFICATION AND TRAINING OF PERSONNEL

This section outlines the minimum educational and training requirements that should be met by individuals involved in DNA typing. This would include training requirements and examiner certification. Current educational qualifications and training of the examiners and technicians, as well as documentation of examiner certification, should be maintained by the DNA Analysis Unit.

DOCUMENT CONTROL

These guidelines establish proper specimen criteria and chain of custody procedures for accepting specimens for DNA typing. Other situations involving chain of custody procedures will follow established laboratory policies. Directions for proper recording of the analytical data and the use of specific report forms are detailed in the QC manual. Separate documentation should be maintained for equipment used in the DNA analysis. These records should describe all maintenance performed on the equipment as well as any instrument calibrations performed by the manufacturer. Where appropriate, records of daily equipment calibrations should also be maintained in order to demonstrate instrument reliability. Batch or lot numbers of all materials considered critical to DNA typing should be recorded according to established criteria.

CONTROL OF EQUIPMENT AND MATERIALS

A listing of specific reagents and suppliers will be maintained. Standard operating procedures for DNA typing will be prepared and will include procedures for the preparation of reagents and reference standards. The SOP should also include, where necessary, instructions for instrument calibration and schedules for routine annual maintenance service.

DNA TYPING

To ensure that both the known liquid blood samples and questioned stains are treated identically through out the process, and to facilitate handling of the specimens within the laboratory, stains should be prepared from liquid blood samples prior to analysis. Other biological materials submitted for DNA testing will be made into stains when appropriate.

One of the most important internal quality controls used in the DNA typing protocol is the use of a human cell line. This internal control is treated in an identical manner as the known and questioned stains, and it serves as both an overall system control and a check of the efficiency of the DNA recovery process. This human cell line produces a distinctive pattern of DNA fragments which is defined by the specific restriction enzyme and probe used. The final development of the expected human cell line DNA pattern verifies that neither the reagents or treatment of the stain have altered the performance of the analytical procedure.

In addition to the human cell line, the performance of DNA typing is monitored through the use of specific QC standards that are integrated into various steps of the assay.

The quantity and quality of the DNA product obtained from the extraction and purification process is monitored electrophoretically by the appearance of high molecular weight DNA. The amount of extracted, high molecular weight DNA can be estimated through the use of DNA standards of known quantities carried on the same gel.

The quality and efficiency of the restriction enzyme digestion is monitored by several means. Each lot of restriction enzyme is tested against a defined source of purified lambda DNA. Before the restriction enzyme is accepted for use, both the lambda DNA and the human cell line DNA must consistently produce a specific band pattern following digestion. Further, an aliquot of the post digested DNA mixture from each specimen is analyzed electrophoretically to determine the completeness of the digestion process.

Once it has been determined that the restriction enzyme has properly digested the DNA, the DNA fragments are separated electrophoretically into discrete bands using

an analytical gel. This allows for a further assessment of the digestion process through visual inspection of the analytical gel. Each analytical gel carries three different internal controls that allow monitoring of the separation process. These controls include: (1) DNA markers of known size applied at different positions in the gel; (2) bromophenol blue tracer in all samples and (3) the human cell line DNA.

Prior to transfer of the DNA fragments from the analytical gel to the nylon membrane by Southern blotting, each lot of membrane has been evaluated to ensure that the membrane is capable of binding an effective level of DNA. In addition to the internal QC procedures just noted, an external QA program would also be run consisting of open and blind proficiency test samples that would be submitted periodically to the DNA Analysis Unit for analysis. The open proficiency specimens would consist of a variety of biological materials of known DNA phenotypes. These specimens would be submitted to the unit on a regular basis. The blind proficiency test specimens would also be prepared from biological materials of known DNA phenotypes. The blind specimens would be submitted to the DNA Analysis Unit in such a manner as to appear as actual evidentiary stains. The exact number of open and blind proficiency samples submitted would be a percentage of the number of actual case specimens submitted for analysis.

DATA AND METHODS VALIDATION

Research reports and publications involving the validation of the data and methods used in DNA typing will be maintained in an area that is readily accessible to the appropriate personnel.

SAFETY

Laboratory personnel will be provided training in general laboratory safety, and written safety guidelines will be distributed to all laboratory workers. A radiation safety officer will be designated for the FBI Laboratory. Guidelines for the safe handling and disposal of biological and radioactive waste will be prepared and made available.

AUDITS

The final element of a QC program is the audit (review). The audit of the QC and DNA protocols should be conducted at least once a year by experienced personnel from outside of the DNA Analysis Unit. The auditors would compare actual laboratory practices to those presented in the SOP, thereby providing a method for verifying that the elements of the QC program, as well as the specific DNA assay requirements, are being met. The audits should be scheduled in advance and with the knowledge and participation of the individuals whose work is being audited.

CONCLUSION

The implementation of DNA typing in the FBI Laboratory marks an important advancement in forensic science. Because of the complexity of the technique and the impact of the data, it is essential that the procedure be scientifically sound and that the quality of the analytical data generated be maintained. These goals can be met, through implementation of a detailed QA/QC program. This program consists of separate QA and QC functions. The QA is an external function consisting of blind and open proficiency test samples and is used to verify that the QC function is being performed adequately. The QC program is an internal function that ensures that the DNA typing results satisfy established performance criteria.

The combined QA/QC program not only monitors the DNA typing process, but also provides laboratory managers and examiners, as well as the judicial system, with the confidence that the quality of the analytical process and accurate interpretation of the data are maintained.

Table 1: Proposed Quality Control Program for the DNA Typing of Biological Materials.

1. Planning and Organization

- 1.1 Documentation
  - 1.1.1 Scope
  - 1.1.2 Policy
  - 1.1.3 Authority and accountability
  - 1.1.4 Program objectives

2. Qualification and Training of Personnel

- 2.1 Qualification records of Examiners and technicians
  - 2.1.1 Educational qualifications
  - 2.1.2 Training received
  - 2.1.3 Certification (Examiners)

3. Document Control

- 3.1 Guidelines for preparation of case reports
- 3.2 Reagent and equipment logs
- 3.3 Chain of custody procedures

4. Control of Equipment and Materials

- 4.1 Procurement
  - 4.1.1 Reagents sources and suppliers
- 4.2 Reagents and reference standards
- 4.3 Instrument calibration and maintenance

5. DNA Typing

- 5.1 Assay procedures
  - 5.1.1 Stain extraction
  - 5.1.2 Purification of DNA
  - 5.1.3 High molecular weight test gels
  - 5.1.4 Restriction enzymes
  - 5.1.5 Restriction enzyme test gels
  - 5.1.6 Analytical gels
  - 5.1.7 Southern blots
  - 5.1.8 Hybridization
  - 5.1.9 Autoradiography
- 5.2 External quality control
  - 5.2.1 Open proficiency testing
  - 5.2.2 Blind proficiency testing

6. Data and Methods Validation

7. Safety

- 7.1 General laboratory safety guidelines
- 7.2 Radiation safety guidelines
- 7.3 Disposal of biological and radioactive waste

8. Audits

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Feature Article

FBI Laboratory DNA Evidence Examination Policy

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The FBI Laboratory's DNA Analysis Unit is now prepared to accept physical evidence for deoxyribonucleic acid (DNA) testing. Application of this technology to forensic evidence is the result of 2 years of research by scientists at the FBI's Forensic Science Research and Training Center, Quantico, Virginia, resulting in the development of the DNA analysis method, known as restriction fragment length polymorphism (RFLP). This method has the capability of absolutely identifying an individual as the source of a body fluid stain. Extensive validation studies of the RFLP technique as well as studies to determine the effects of the environment, contaminants, aging and the mixing of body fluids on DNA and forensic stains have been completed.

Since the FBI Laboratory is presently the only police crime laboratory in the United States with this capability, a significant increase in case submissions is anticipated. In order for the FBI Laboratory to be responsive to this increase, and considering its limited resources, a selective case acceptance policy has been established to efficiently handle these requests and to effectively utilize the potential of DNA analysis to its fullest extent.

In general, this policy states that the FBI Laboratory will accept evidence for DNA analysis from current, violent personal crimes where appropriate standards for comparison are available. Specifically, DNA analysis for state and local cases will be limited to homicide, sexual assault and serious aggravated assault cases in which a suspect has been identified. In certain cases, evidence will be accepted by the FBI Laboratory for DNA analysis even though a suspect has not been identified. These exceptions include serial homicide/rape cases and sexual assaults on young children.

Other factors of the FBI Laboratory DNA evidence examination policy are as follows:

Known blood samples from both the victim and suspect are required for comparison purposes.

Requests for DNA analysis on previously adjudicated cases should not be submitted to the FBI Laboratory, but should be referred by the investigating agency to one of the private DNA testing laboratories. Names and addresses of these laboratories can be provided on request.

DNA analysis will not be conducted on any evidence which has been previously subjected to DNA analysis by another laboratory, except in cases where there is overwhelming justification for such a reexamination, and it would be in the best interest of the criminal justice system.

No examination will be conducted on evidence which has been previously examined by another expert. However, the FBI Laboratory will accept samples for DNA analysis which have been previously tested serologically by state and local laboratories not having DNA capabilities. Examination of these specimens is contingent on a determination of the suitability (that is, size, quality and probative value) of the sample for DNA analysis. The local crime laboratory should contact the DNA Analysis Unit of the FBI Laboratory prior to submission of this kind of evidence.

Law enforcement agencies requesting DNA analysis are encouraged to submit evidence to their local crime laboratory for traditional serological testing prior to the submission of samples to the FBI Laboratory for DNA testing.

Body fluid stained evidence submitted for serological and/or DNA analysis should be completely air-dried before packaging and submitted promptly to the FBI Laboratory. Blood samples from the suspect and victim should be collected by medical personnel in two vacutainer tubes, one containing EDTA for DNA analysis and the other containing no preservative for serological analysis. These blood samples should be submitted to the FBI Laboratory without delay. In the event there will be a delay in the submission of the dried stain evidence to the FBI Laboratory, it should be kept frozen. Inquiries regarding the submission of evidence to the FBI Laboratory for DNA analysis or the case acceptance policy should be directed to the FBI Laboratory's DNA Analysis Unit, telephone (202)324-5436.

Feature Article

Summary of the First Meeting of the Technical Working Group on DNA Analysis Methods

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During November 20-22, 1988, the FBI hosted the first meeting of the Technical Working Group on DNA Analysis Methods (TWGDAM). The purpose of this first meeting was: (1) to pull together a select number of individuals from public forensic laboratories who are actively implementing DNA analysis methods; (2) to discuss the methods now being used or researched; (3) to compare the work that has been done to date and; (4) to share protocols where appropriate.

SESSION ONE

Presentations were made by Dr. F. Samuel Baechtel, Forensic Science Research Unit, FBI Laboratory; Mr. Terry Laber, Minnesota Bureau of Criminal Apprehension and Mr. Richard Guerrieri, Virginia Tidewater Regional Crime Laboratory on the aspects of three DNA recovery and quantification methods. The methods discussed were: phenol/chloroform/alcohol precipitation method (Baechtel); Non-organic/high salt precipitation method (Laber) and phenol/chloroform/dialysis method (Guerrieri). Each of the methods were shown to have been used successfully to extract DNA from liquid blood, semen, bloodstains and semen stains. Following a general discussion on the advantages and disadvantages of the three methods as well as discussion on other methods being used by the members of the working group, it was agreed upon that a small study would be conducted to measure the efficiency of the various DNA recovery methods. The study will include the extraction of two standard panels of dried stains. Each stain panel will consist of individual blood and semen stains. Seven different laboratories agreed to participate in the study.

SESSION TWO

Dr. George Sensabaugh, University of California Berkeley, who acts as the technical advisor to TWGDAM on the polymerase chain reaction (PCR) dot blot method gave a report on the current status of the PCR HLA DQ-Alpha dot blot method and its application to forensic science.

In his presentation Dr. Sensabaugh described the new membranes being produced by the Cetus Corporation having fixed allele-specific oligonucleotide probes (ASO). He further described the successful use of the PCR HLA DQ-Alpha dot blot method in the analysis of evidentiary material from rape and homicide cases and in matters involving limited biological material.

Dr. Catherine Comey, Forensic Science Research Unit, FBI Laboratory, reviewed with the group the work which the FBI Laboratory has done on the PCR HLA DQ-Alpha dot blot method. The FBI Laboratory has successfully amplified the DQ-Alpha gene from DNA from liquid blood, bloodstains, semen, semen stains, forcibly removed hair and telogen hairs, and the fractions representing semen and vaginal cells that has been differentially extracted from vaginal swabs. Dr. Comey discussed the difficulty she has had with the amplification of DNA from bloodstains following initial purification. Dr. Comey is also evaluating the fixed probe membranes and the DQ-Alpha subtyping probes. It is her opinion that the fixed probe membranes will be difficult to handle in those laboratories analyzing a large volume of samples.

Following some general discussion on the PCR dot blot methodology the areas that follow were singled out as suggested areas needing additional research and development:

(a) evaluation of fixed probe membranes for forensic use.
(b) additional evaluation on forensic materials and population studies using the PCR HLA DQ-Alpha dot blot.
(c) PCR amplification and sequencing of mitochondrial DNA.
(d) feasibility of using PCR amplification

of RFLPs and their application to forensic DNA analysis.

(e) additional work on the extraction and amplification of DNA from shed hairs.

The research work on the suggested areas would center, out of necessity, in the Royal Canadian Mounted Police (RCMP), FBI and California laboratories.

SESSION THREE

Ms. Margaret Kuo, Orange County Crime Laboratory, California, reviewed with the group the results of a blind trial proficiency test administered to the Lifecodes Corporation, Cetus Corporation and Cellmark Diagnostics by the California Association of Crime Laboratory Directors (CACLD). Results of the study can be obtained directly from CACLD (DNA Committee Report #6).

In addition, Mr. James Mudd, Forensic Science Research Unit, FBI Laboratory, presented a draft proposal on a quality control/quality assurance plan for a DNA analysis laboratory.

Following some discussion from the groups concerning various controls used in conducting DNA analysis it was proposed that a committee be formed from the members of the TWGDAM to formulate a QC/QA program for DNA analysis laboratories. A draft of the plan is to be completed and presented to TWGDAM members in their next meeting. Members of the committee included the following:

Mr. James Mudd, FBI Laboratory
Ms. Margaret Kuo, Orange County Crime Laboratory, California
Mr. Willard Stuver, Miami-Dade Crime Laboratory, Florida
Mr. Mark Nelson, North Carolina Bureau of Investigation
SA Larry Presley, FBI Laboratory

SESSION FOUR

Presentations were made by Dr. John Waye, RCMP, Central Forensic Laboratory; Dr. Bruce Budowle, Forensic Science Research Unit, FBI Laboratory; and Ms. Jacqueline Emrich, Virginia Tidewater Regional Crime Laboratory. Each presenter discussed the restriction fragment length polymorphism (RFLP) method now under consideration in their laboratory and the work that is being/or has been done to validate the method for use on evidentiary material.

Dr. Waye reported that the RCMP laboratory has chosen HAE III as their restriction enzyme and are running 15 cm analytical gels. They are probing their target DNAs with the VNTR probes, YNH24 (D2S44), CMM101, (D14S13) and 3'HVR and have completed a lengthy population survey involving 700 caucasians. Dr. Waye further advised that the RCMP laboratory has additional population studies underway; is evaluating other VNTR probes, has developed a simple dot blot method for quantifying human DNA and is evaluating the use of PCR in DNA analysis.

Dr. Budowle reported the FBI Laboratory has completed the developmental work on an RFLP method based on the restriction enzyme HAE III and the VNTR probes; YNH24 (D2S44) CMM101 (D14S13), 3'HVR, MS-1 (D1S7) (Cellmark) and V1 (D17S59) (Lifecodes). The analytical gels used in the system are 15 cm and require a 16 hour run time. He stated further that population studies are being completed on 250 caucasians and 240 blacks. A lengthy study measuring the effects of time, light, heat and chemical and biological contaminants on DNA has been completed.

Ms. Emrich reported on the Virginia State Crime Laboratory's successful efforts to evaluate an RFLP method (Lifecodes) based on the restriction enzyme Pst and VNTR probes YNH24 (D2S44) V1 (D17S59) and C1 (DXYS14). She stated the Virginia State Crime Laboratory is presently running a series of proficiency samples provided by Lifecodes. Ms. Emrich advised that her laboratory will continue to evaluate the Lifecodes method by running a variety of different types of samples over the next six months. The Virginia Crime Laboratory continues to target the late Spring of 1989 to implement DNA typing in their laboratory.

Questions raised during the discussion period on RFLPs included: (1) the value of long analytical gels/long run time versus short analytical gels/short run time, (2) resolution of DNA fragments on long versus short analytical gels, (3) the cost of certain reagents such as the use of polyethylene glycol versus dextran sulfate in the hybridization solutions, (4) the

cost and availability of probes from the private laboratories, (5) the availability and suitability of size markers, and (6) the choice of restriction enzyme. It was apparent to the group from the work presented by each laboratory that successful DNA analysis could be obtained from more than one RFLP method. Dr. Waye agreed to run the FBI Laboratory system and report his results to the group at the next meeting.

SESSION FIVE

Dr. Dwight Adams, DNA Analysis Unit, FBI Laboratory, reviewed his work on the effects of time, heat, light, chemical and biological contaminants on DNA analysis. A summary of the results of Dr. Adams' work appears on page 106-108 in this issue. It was apparent, however, that although environmental factors will effect the forensic serologist ability to obtain DNA typing from some types of specimens they do not effect DNA in a way to produce improper typing or false positives or negatives.

Dr. Keith Monson, Forensic Science Research Unit, FBI Laboratory, presented the results of his work in the development of a computer assisted reader for the analysis of DNA patterns obtained from VNTR RFLP analysis. Dr. Monson has developed the software for an PC/AT based system for reading autoradiograms obtained from VNTR RFLP analysis. The system takes approximately two minutes to read a single autoradiogram having 14-20 samples. It is the intent of the FBI Laboratory to make the software for the system available to the forensic science community in the near future. Dr. Monson also discussed the FBI Laboratory's progress in the development of the software and hardware for a national DNA profiling database.

In summary the first meeting of the Technical Working Group on DNA Analysis Methods provided a forum to exchange ideas and information relating to the DNA analysis methods now being implemented in the various public forensic laboratories. Protocols were shared between the participants, where permitted, with the intent that the various participants would run the different protocols in their laboratories and report the results back at the next TWGDAM meeting scheduled in February 1989.

Meeting Announcements

REGIONAL FORENSIC SCIENCE ASSOCIATION MEETINGS

The Southern Association of Forensic Scientists (SAFS) will hold its Spring 1989 meeting at the Radisson Plaza Hotel in Raleigh, North Carolina from May 4-6, 1989. The theme of this meeting is "Scanning Electron Microscopy in Forensic Science."

For further information, contact Program Chairman Lieutenant Robert S. White, West Virginia State Police Laboratory, 725 Jefferson Road, South Charleston, West Virginia 25309 (telephone: 304-746-2181).

The Northwest Association of Forensic Scientists (NWAFS) will hold its Spring 1989 meeting at the Ashland Hills Inn, Ashland, Oregon from April 3-7, 1989. In addition to workshops and presentations, the meeting agenda will include tours of the new National Fish and Wildlife Forensic Laboratory in Ashland.

For further information contact, program chairman, Beth Ann Gilroy, National Fish and Wildlife Forensics Laboratory, 1490 East Main Street, Ashland, Oregon 97520 (telephone 503-482-4191, FAX 503-482-4989). Deadline for abstracts and/or registration is February 10, 1989.

NATIONAL FORENSIC SCIENCE ASSOCIATION MEETINGS

The 41st Annual Meeting of the American Academy of Forensic Sciences (AAFS) will be held at the Riviera Hotel in Las Vegas, Nevada from February 13-18, 1989.

For further information, contact AAFS, P.O. Box 669, Colorado Springs, Colorado 80901-0669 (telephone: 719-636-1100).

The 20th Anniversary Seminar of the Association of Firearms and Tool Mark Examiners (AFTE) will be held at the Virginia Beach Resort and Conference Center, Virginia Beach, Virginia from April 23-29, 1989. The training program will include presentations in the following subject areas: comparison microscope techniques and procedures, tool marks as they relate to locks and safes, wounds and wound ballistics, conversion of semi-auto firearms to full-auto mode of fire, and techniques and procedures in the use of photography.

Technical papers are requested for presentation at this seminar. Submit abstracts by October 1, 1988 to Ann D. Jones, Bureau of Forensic Science, P. O. Box 999, Richmond, Virginia 23208 (telephone: 804-786-4706). For further information on the seminar, contact Conference Chairman John G. Ward, Tidewater Regional Forensic Laboratory, 401-A Colley Avenue, Norfolk, Virginia 23507-1966 (telephone: 804-623-8327).

The 42nd Annual Meeting of the American Academy of Forensic Sciences (AAFS) will be held at the Clarion Hotel in Cincinnati, Ohio from February 19-24, 1990.

For further information, contact AAFS, P.O. Box 669, Colorado Springs, Colorado 80901-0669 (telephone: 719-636-1100).

INTERNATIONAL FORENSIC SCIENCE ASSOCIATION MEETINGS

The Laboratory Division of the Federal Bureau of Investigation will host a symposium entitled "An International Symposium on the Forensic Aspects of DNA Analysis" from June 18-23, 1989 at the FBI Academy in Quantico, Virginia. This symposium will provide a forum for the discussion of techniques and issues relevant to the forensic aspects of DNA analysis. Plenary sessions will be devoted to lectures by prominent scientists on topics such as restriction fragment length polymor- phisms, polymerase chain reaction, extraction methods, blotting techniques, mitochondrial DNA, allele specific probes, sequencing and population genetics. Applications and abstracts should be forwarded by December 1, 1988 to the

Symposium Coordinator, FSRTC, Room 212, FBI Academy, Quantico, Virginia 22135 (telephone: 703-640-1123).

For further information, contact the Symposium Coordinator.

The 103rd Annual International Meeting and Exposition of the Association of Official Analytical Chemists (AOAC) will be held at the Clarion Hotel in St. Louis, Missouri from September 25-28, 1989.

For further information, contact Margaret Ridgell, AOAC, 1111 North 19th Street, Suite 210, Arlington, Virginia 22209.

The 13th International Congress of the International Society for Forensic Hemogenetics will be held in New Orleans, Louisiana from October 18-20, 1989.

For further information contact Dr. Herbert Polesky, Memorial Blood Bank Center, 2304 Park Avenue South, Minneapolis, Minnesota 55404.

The Pan American Association of Forensic Sciences will hold its fourth international meeting in Bogota, Columbia in November, 1989. The theme of this meeting is "The Sciences and Justice."

For further information, contact Dr. Egon Lichtenberge, Carrera 11 A 96-26, Bogota, Columbia.

The 12th Meeting of the International Association of Forensic Sciences (IAFS) will be held from October 24-31, 1990 in Adelaide, South Australia. The theme of this meeting will be "Towards a Professional Profession."

For further information, contact IAFS 1990, P.O. Box 753, Norwood, South Australia 5067 (telephone: 08-227-0644).

Instructions for Submitting Articles

In order to facilitate rapid publication, submissions should conform to the following:

1. Manuscripts should be typed, double-spaced, on 8 1/2" x 11" paper. Submit three copies of the manuscript (one of which must be the original with glossy photographs).

2. Tables should be as simple as possible. Each table should be typed on a separate sheet of paper and should include a heading.

3. Graphs and diagrams should be submitted as sharp photographs on white glossy paper.

4. References in the body of the paper should refer to the author's (authors') name(s) and the date of publication. For example:

   According to Doe and Smith (1972)....

   ....has been discussed in the literature (Doe and Smith 1972; Weinburger et al. 1984; Jones 1981).

5. References at the end of the paper should appear in alphabetical order. Journal references should include the author's (authors') name(s), date of publication, title of article, journal name, volume number, and the beginning and ending pages of the article. Please use the following format:

   Davis, H. E. and Jones, B. A. (1978). Seasonal variations in plasma hormones, Biol. Reprod. 88:271-273.

   List articles or chapters within a book as follows:

   deHaseth, J. A. (1982). Fourier transform infrared spectrometry. In: Fourier, Hadamard and Hilbert Transformations in Chemistry, (Marshall, A. G., ed.) Plenum Press, New York, pp. 387-420.

6. Technical papers should be written in an Introduction, Materials and Methods, and Results and Discussion format.

7. When reference is made to a particular product, state the manufacturer of the product and the city and state of the manufacturer's headquarters. For example:

   Isotactic PS (Dow Chemical, Midland, MI)....

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*Note: The S following the page number is from Volume 15, Supplement 1, 1988

DNA Profiling
A Tool for Law Enforcement

"Advances in DNA technology represent perhaps one of the most significant forensic breakthroughs of the century. . . ."

By
JOHN W. HICKS, M.P.A.
Deputy Assistant Director
Laboratory Division
Federal Bureau of Investigation
Washington, DC

Consider the following scenarios:

—Police investigating the brutal slaying of a young woman in a southeastern town carefully collect physical evidence at the scene and submit it to their local crime laboratory. Forensic analysis reveals the presence of semen from which a DNA identification profile is determined. This profile is searched through a computerized data bank and a "hit" is made with DNA profiles from similar crimes which occurred months earlier in two northeastern cities. Investigators from these jurisdictions share investigative data, and a suspect is developed. A blood sample obtained from the suspect reveals the same DNA profile, which conclusively identifies him with the semen recovered from the three murder victims.

—The partially decomposed body of a child is found in a rural area. From samples of hair and tissue taken from the remains, a DNA identification profile is made for comparison with DNA profiles of parents of reported missing children.

—Semen is identified on swabbings taken from a rape victim by the attending physician and submitted for analysis, which results in a DNA identification profile being developed. The profile is searched through a central DNA profile data bank and a "hit" is made. The rapist is identified as a resident of an adjacent State who had been convicted 3 years earlier on a burglary charge.

Recent breakthroughs in DNA technology are expected to provide investigators with powerful forensic tools to help solve these difficult kinds of cases.

Personal identification has always been of vital concern to law enforcement. In support of this need, the crime laboratory's primary mission is to apply science to develop information from material recovered from a crime scene, which will identify the perpetrator or associate the perpetrator with the victim or the scene of the crime.

(Published by the Federal Bureau of Investigation, U.S. Department of Justice)
Reprinted from the FBI Law Enforcement Bulletin, August, 1988.

Deputy Assistant Director Hicks

The evidentiary materials most frequently recovered in the investigation of a violent crime, such as homicide or rape, are blood, hair, and semen. According to a study conducted in 1984, investigators in many jurisdictions recover these types of evidence more frequently than fingerprints.[1] However, until recently, the forensic analyst has been able to make only limited associations using biological material. DNA technology now provides the analyst with the ability to identify a particular individual based on a drop of blood or semen, or a single hair. Because of its capability to individualize, it is very often referred to as "DNA fingerprinting."

What is DNA?

Deoxyribonucleic acid (DNA) is an organic substance found primarily in the nucleus of living cells. It comprises the chromosomes within the nucleus and provides the genetic code which determines a person's individual characteristics. The code is expressed by the arrangement of four basic building blocks, called nucleotides, which are represented by the letters A (adenine), G (guanine), C (cytosine), and T (thymine). These nucleotides are linked in chain-like sequences, and their order can vary to provide an almost infinite number of possible arrangements. There are about 3 billion nucleotides in the entire human genetic code.

One of the techniques scientists use to characterize the DNA found in body fluids and tissue specimens is referred to as restriction fragment length polymorphism (RFLP). Special proteins are used to cut the DNA being analyzed at specific sites. These proteins are called restriction enzymes and recognize specific short sequences of four to eight nucleotides, referred to as restriction sites. The restriction sites of greatest value to the forensic scientist are those which are highly variable in the human population (polymorphic). This cutting process results in fragments of DNA of various lengths; hence, the name restriction fragment length polymorphisms. These fragments are then separated on a gel-covered glass plate by a process called electrophoresis. By using other pieces of DNA of known sequence called probes, the analyst can identify the locations on the plate of the DNA fragments of interest. This typically results in a pattern of bands which can then be transferred to photographic film to be interpreted by the analyst.

This a very brief description of the DNA testing process, and there are other methods which are used to characterize DNA.[2] Many different restriction enzymes and DNA probes can be used, and each results in a different banding pattern and provides individual discriminating powers of different values. Because of these variations in test procedures, scientists are now unable to compare test results directly. The ability to classify such information, catalog it, and later search it against other test results is critical for law enforcement use.

The FBI Role

The FBI has initiated an aggressive forensic research program to develop this technology for eventual implementation in the FBI Laboratory. An ambitious technical training program

"A national coordination effort is essential if the full law enforcement potential of DNA technology is to be realized."

is also being developed to instruct personnel of State and local crime laboratories throughout the United States on the use of this technology. The FBI research effort is directed not only at methods development but also at establishing the scientific validity and reliability of these methods to insure that evidence derived from the forensic analysis of DNA can withstand legal challenges to its introduction in the courts.

DNA technology is expected to impact substantially not only the crime laboratory in implementing the technique but also the way certain types of violent crimes are investigated. Classifying systems are now being explored which will allow the DNA profile identifying information to be entered into a centralized computer data bank.

As in the situations described at the beginning of this article, semen collected from victims of unknown subject sexual assault cases might be analyzed and the DNA profiles compared with similar cases stored in the data bank. This would permit definitive linking of similar crimes in one or several jurisdictions, which might not otherwise appear related, thereby facilitating the coordination of leads and other investigative information.

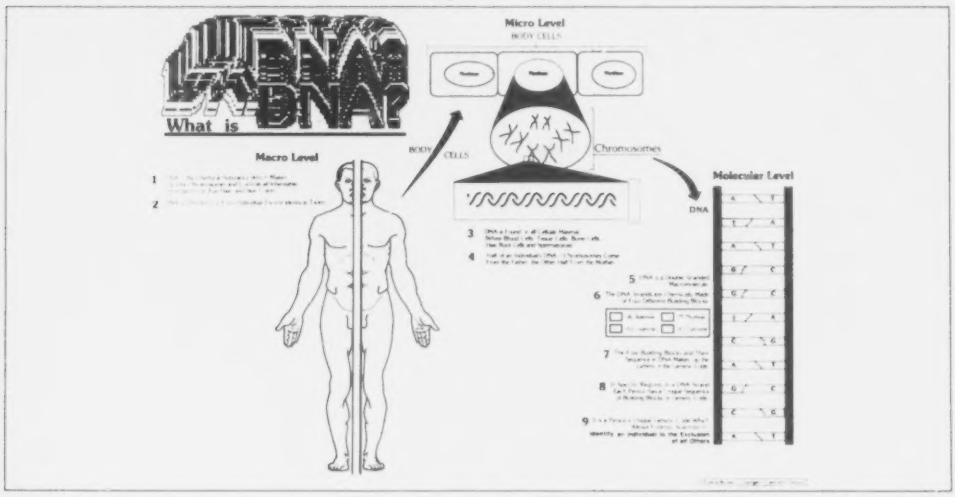
Legislation has been proposed in a few jurisdictions which provides for blood samples to be taken for genetic typing from convicted sex offenders as a condition of paroled release. Such a file would provide a reference against which blood, semen, or hair from the scene of a subsequent crime might be searched. There is also the potential for the establishment of civil files containing voluntarily furnished DNA profiles of individual citizens which might be used to aid in the identification of human remains at the site of a mass disaster.

DNA profiling would be useful to the medical examiner tasked with the identification of unidentified remains. The deceased's DNA profile might be retained for comparison with DNA profiles of the parents or children of missing persons to accomplish an identification through paternity-type DNA testing.

Each of the above concepts, as well as others which might be developed for the application of DNA technology to law enforcement problems, would require the understanding, interest, and support of the user community. The user community is broad and includes crime laboratories, police departments, sheriff's offices, medical examiner's offices, district attorney's offices, and others. A national coordination effort is essential if the full law enforcement potential of DNA technology is to be realized.

Implementation Strategy

A seminar on DNA technology held in June 1988, at the Forensic Science Research and Training Center at the



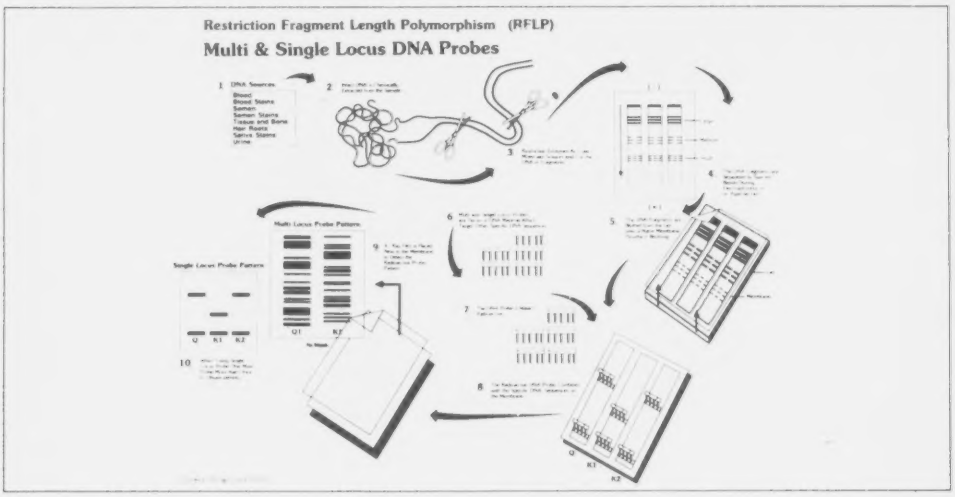
"A national commitment to the routine use of DNA technology by police agencies at all levels will result in a more efficient and effective law enforcement system."

FBI Academy in Quantico, VA, was attended by key forensic and medical researchers from academia, the private sector, and the international crime laboratory community. Two important topics addressed at the meeting were the establishment of DNA standards within the forensic science community and the utility and feasibility of automated data files containing DNA identification profile information. Clearly, it is advantageous to law enforcement on a national scale to coordinate the development of these kinds of systems with the establishment of appropriate controls and standards to permit the effective exchange of DNA identification profiles. To accomplish this, the community must agree upon standards which provide a common language and reference bases to facilitate the exchange of critical investigative information. The system must at the same time permit flexibility to accommodate changes as DNA technology continues to evolve. The success of this effort will depend heavily on strong professional commitment to the needs of law enforcement, along with a spirit of cooperation and mutual support within the forensic community.

Steady progress is being made in transferring this important technology to the crime laboratory community. In April 1988, the FBI extended invitations to directors of crime laboratory systems throughout the United States, requesting them to nominate individuals to participate in a visiting scientist program at the Forensic Science Research and Training Center. This 4-month program is designed to provide the technical resources to address the validity and reliability issues associated with DNA testing as quickly as possible. Upon completion of a research project, worked in collaboration with FBI scientists, participants will have developed technical proficiency in the test methods and will have obtained valuable experience in applying DNA implementation efforts in their individual State and local laboratories. A specialized technical training course is being developed for State and local laboratory personnel which will be offered in the fall of 1988. This course, in conjunction with other studies, will also facilitate the introduction of DNA test methods at the local level.

It is anticipated that within 2 to 3 years, DNA testing will be performed fairly routinely on evidence samples. There is an extremely high level of interest in this technology, and if the demand is to be satisfied, agency administrators must take the necessary action to provide the necessary technical personnel and equipment resources.

When forensic DNA testing was initially introduced into this country, the tests were performed at only a few private testing laboratories. During the transition period, as the technology is



transferred to the Nation's crime laboratories, there will continue to be only a limited number of facilities capable of performing these tests. This will necessitate a high degree of selectivity as to which evidence samples are submitted for DNA analysis. Considerations might include an assessment of the probative value of the item of evidence, the size and condition of the evidence stain or specimen, and possible delays which might result in the judicial proceedings while waiting for test results. A physical evidence evaluation and appraisal may show that traditional forensic testing can provide sufficient information that would preclude the need for DNA tests.

Considerations

While DNA technology will provide a powerful new capability for law enforcement, it will not necessarily displace the forensic methods now employed in the crime laboratory. Not all biological specimens will be suitable for DNA testing, or tests conducted may not provide a conclusive result. In these situations, classical serological tests will still be necessary to glean all possible probative information from the evidence materials. Because of the limited resources available for DNA testing, standard serology tests may be used to screen evidence samples to select the materials most likely to provide a successful DNA result. There will remain a need to identify, isolate, preserve, and analyze a wide range of evidence types, such as firearms, toolmarks, textile fibers, paint, and glass, as well as biological specimens to provide additional information to aid in crime reconstruction.

Care should be exercised by investigators in processing crime scenes to insure other evidence types are not overlooked in the hope of a definitive DNA test result. It is recommended that the local crime laboratory be consulted and kept involved in the evidence evaluation process throughout the investigation. These forensic specialists will insure other evidence types are appropriately analyzed and can assist in isolating materials for DNA testing, even though they may not now perform the test in their laboratories.

National implementation of DNA testing on evidence samples will require law enforcement agencies throughout the United States to commit additional resources for forensic services. Because of the complexity of DNA technology and the nature of the testing process, it is advisable that technical personnel be identified who can be dedicated to DNA testing. This will encourage a high level of technical proficiency and facilitate effective quality control procedures. Start-up costs for DNA testing include some specialized laboratory equipment, and in some instances, acquiring the necessary laboratory space to perform the tests. As testing gets underway, there will be additional costs for the chemical reagents used in the process and the DNA probe materials. Sources in the private sector have been identified who will provide these supplies, perhaps in the form of testing kits.

Benefits

It is anticipated that the costs associated with the forensic application of DNA testing will be substantially offset by savings in investigative manhours required to develop evidence with which to sustain prosecutions. When this technology is fully implemented, it has the potential to identify perpetrators of crimes sooner in the investigative process and to clear suspects more readily so that investigative resources can be focused more productively. As the criminal justice system becomes better acquainted with the potential power of DNA technology, it is anticipated that additional savings in court time may be realized through shorter trials or averting trial altogether through an increased number of pleas.

Summary

Advances in DNA technology represent perhaps one of the most significant forensic breakthroughs of the century in its ability to identify a rapist or murderer based on trace amounts of biological evidence left at the scene of a crime.

—The goal of making DNA profiling a part of a crime laboratory's arsenal of scientific investigative techniques is being realized. A national commitment to the routine use of DNA technology by police agencies at all levels will result in a more efficient and effective law enforcement system.

Footnotes

[1] Joseph L. Peterson, et al. *Forensic Evidence and the Police: The Effects of Scientific Evidence on Criminal Investigations*, October 1984, U.S. Government Printing Office, publication 0-461-539:23742.

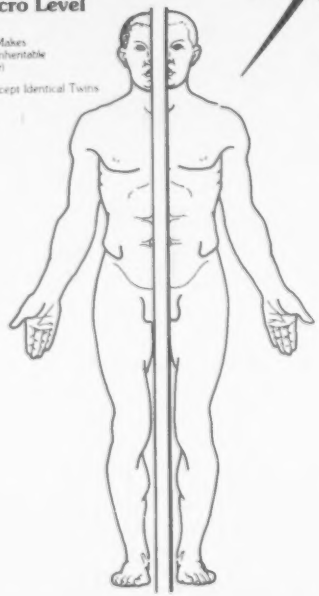
[2] A more-detailed description of the several DNA analysis techniques (written for the nonscientist) can be found in the *Crime Laboratory Digest*, 1988, vol. 15, supplement No. 1, entitled "A Primer on the Methods Used in the Typing of DNA." This publication is available through libraries or by requests directed to the FBI Laboratory, Washington, DC.

What is DNA?

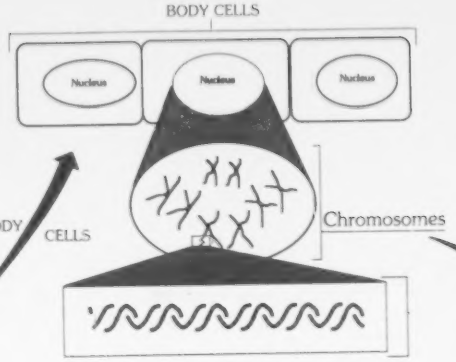
Macro Level

1 DNA is the Chemical Substance Which Makes up Our Chromosomes and Controls all Inheritable Information (i.e. Eye Hair, and Skin Color)

2 DNA is Different for Every Individual Except Identical Twins



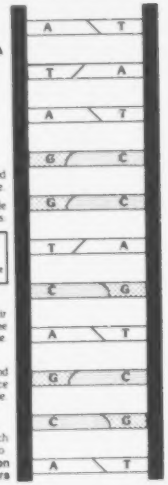
Micro Level



3 DNA is Found in all Cellular Material (White Blood Cells, Tissue Cells, Bone Cells, Hair Root Cells and Spermatozoa)

4 Half of an Individual's DNA / Chromosomes Come From the Father, the Other Half From the Mother.

Molecular Level



5 DNA is a Double Stranded Macromolecule.

6 The DNA Strands are Chemically Made of Four Different Building Blocks

(A) Adenine
(T) Thymine
(G) Guanine
(C) Cytosine

7 The Four Building Blocks and Their Sequence in DNA Makes up the Letters of the Genetic Code.

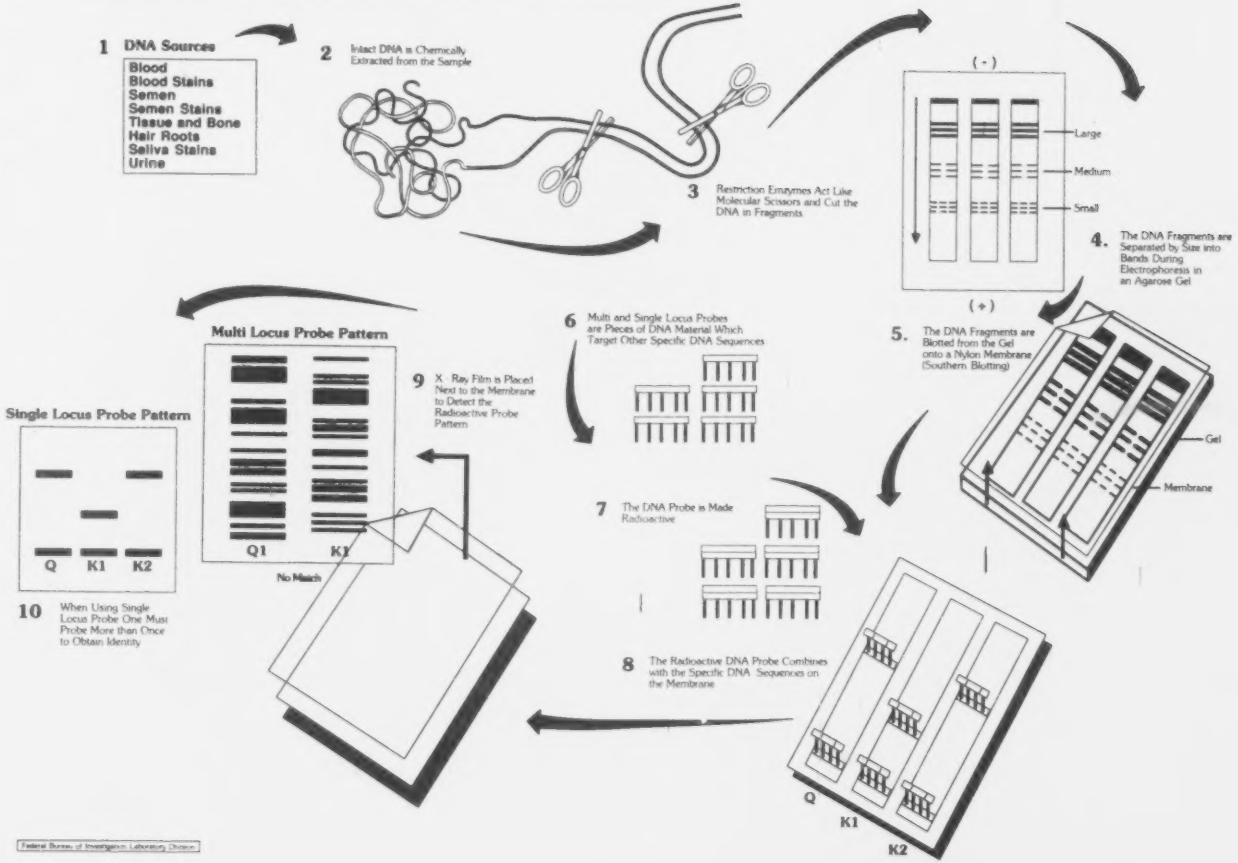
8 In Specific Regions on a DNA Strand Each Person has a Unique Sequence of Building Blocks or Genetic Code.

9 It is a Person's Unique Genetic Code Which Allows Forensic Scientists to **Identify an Individual to the Exclusion of all Others**

Federal Bureau of Investigation Laboratory Division

Restriction Fragment Length Polymorphism (RFLP)

Multi & Single Locus DNA Probes



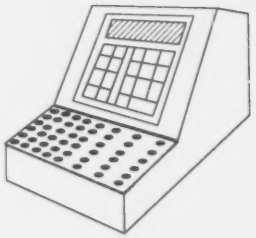
Polymerase Chain Reaction (PCR)

Dot Blot Method

1 DNA Sources

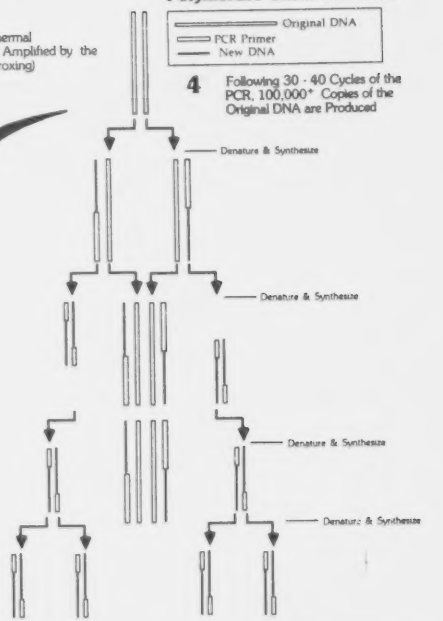
Blood	0.1 ul
Blood Stain	1 ul
Semen	0.5 ul
Semen Stain	1 ul
Hair Root	1-2 Hairs
Tissue	10 ng
Bone	50 ng
Saliva	5 ul
Urine	20 ul

2 Intact or Fragmented DNA is Chemically Extracted



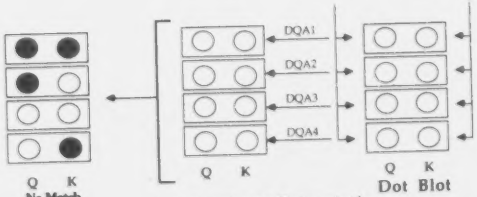
3 DNA is Placed in Thermal Cycle Analyzer and Amplified by the PCR (Molecular Xeroxing)

Polymerase Chain Reaction



4 Following 30 - 40 Cycles of the PCR, 100,000+ Copies of the Original DNA are Produced

5 The Amplified DNA is Immobilized by Spotting it onto a Membrane



6 The Membrane is Challenged with DNA Probe Specific to the HLA DQ-Alpha Allele. The Probe is Conjugated to Horseradish Peroxidase.

7 The Membrane is Washed and Floated in a Color Developer Containing H^2O^2+ Tetramethyl Benzidene

8 There are 21 Different Phenotypes at HLA DQ Alpha Locus (Most Frequent is 1 in 5, Least is 1 in 800) The PCR Dot Blot Method DOES NOT PROVIDE IDENITY

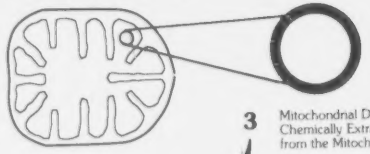
Federal Bureau of Investigation Laboratory Division

Mitochondrial DNA and DNA Sequencing

1 DNA Sources

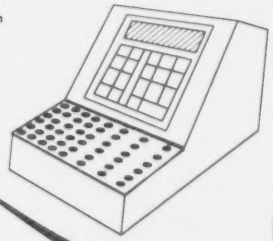
Spermatozoa
Tissue Cells
White Blood Cells
Hair Root Cells

2 Mitochondria are Recovered from Cells by Centrifugation



3 Mitochondrial DNA is Chemically Extracted from the Mitochondria

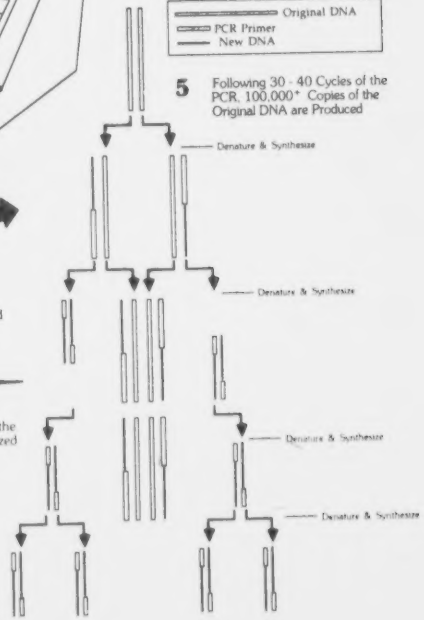
4 Mitochondrial DNA is Placed in Thermal Cycle Analyzer and Amplified by the PCR (Molecular Xeroxing)



Polymerase Chain Reaction

Original DNA
PCR Primer
New DNA

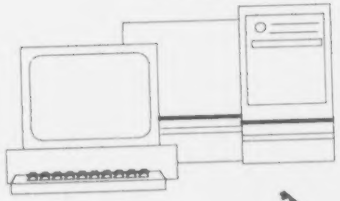
5 Following 30 - 40 Cycles of the PCR, 100,000+ Copies of the Original DNA are Produced



6 Amplified Mitochondrial DNA is Placed in a Chemical Sequencing Reaction

7 The Sequence Products are Placed in the Automated DNA Sequencer and Analzed with Computer Support

DNA Sequencing



8 DNA Having Up to 250 Consecutive Units Can be Sequenced

GGACCTGGAATATGGCGAGAAAACTGAAAATCACGGAAAATGAGA

GGACGTGAAATATGGCGAGAAAACTGAAAAAGGTGGAAAATTTAG

GGACGTGGAATATGGCAAGAAACTGAAAATCATGGAAAAATGAAG

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